200700244

No.

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

Kansas Agricultural Experiment Station

Whereas, THERE HAS BEEN PRESENTED TO THE

Secretary of Agriculture

AN APPLICATION REQUESTING A CERTIFICATE OF PROTECTION FOR AN ALLEGED DISTINCT VARIETY OF SEXUALLY REPRODUCED, OR TUBER PROPAGATED PLANT, THE NAME AND DESCRIPTION OF WHICH ARE CONTAINED IN THE APPLICATION AND EXHIBITS, A COPY OF WHICH IS HEREUNTO ANNEXED AND MADE A PART HEREOF, AND THE VARIOUS REQUIREMENTS OF LAW IN SUCH CASES MADE AND PROVIDED HAVE BEEN COMPLIED WITH, AND THE TITLE THERETO IS, FROM THE RECORDS OF THE PLANT VARIETY PROTECTION OFFICE, IN THE APPLICANT(S) INDICATED IN THE SAID COPY, AND WHEREAS, UPON DUE EXAMINATION MADE, THE SAID APPLICANT(S) IS (ARE) ADJUDGED TO BE ENTITLED TO A CERTIFICATE OF PLANT VARIETY PROTECTION UNDER THE LAW.

NOW, THEREFORE, THIS CERTIFICATE OF PLANT VARIETY PROTECTION IS TO GRANT UNTO THE SAID APPLICANT(S) AND THE SUCCESSORS, HEIRS OR ASSIGNS OF THE SAID APPLICANT(S) FOR THE TERM OF TWENTY YEARS FROM THE DATE OF THIS GRANT, SUBJECT TO THE PAYMENT OF THE REQUIRED FEES AND PERIODIC REPLENISHMENT OF VIABLE BASIC SEED OF THE VARIETY IN A PUBLIC REPOSITORY AS PROVIDED BY LAW, THE RIGHT TO EXCLUDE OTHERS FROM SELLING THE VARIETY, OR OFFERING IT FOR SALE, OR REPRODUCING IT, OR IMPORTING IT, OR EXPORTING IT, OR CONDITIONING IT FOR PROPAGATION, OR STOCKING IT FOR ANY OF THE ABOVE PURPOSES, OR USING IT IN PRODUCING A HYBRID OR DIFFERENT VARIETY THEREFROM, TO THE EXTENT PROVIDED BY THE PLANT VARIETY PROTECTION ACT. IN THE UNITED STATES SEED OF THIS VARIETY (1) MAY BE SOLD BY VARIETY NAME ONLY AS A CLASS OF CERTIFIED SEED AND (2) SHALL CONFORM TO THE NUMBER OF GENERATIONS SPECIFIED BY THE OWNER OF THE RIGHTS. (84 STAT. 1542, AS AMENDED, 7 U.S.C. 2321 ET SEQ.)

WHEAT, COMMON

'RonL'

In Testimony Whereof, *I have hereunto set my hand and caused the seal of the Plant Variety Protection Office to be affixed at the City of Washington, D.C. this twenty-third day of May, in the year two thousand and seven.*

Attest:

Commissioner
Plant Variety Protection Office
Agricultural Marketing Service

Secretary of Agriculture

REPRODUCE LOCALLY. *Include form number and date on all reproductions*

Form Approved - OMB No. 0581-0055

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL MARKETING SERVICE
SCIENCE AND TECHNOLOGY - PLANT VARIETY PROTECTION OFFICE

APPLICATION FOR PLANT VARIETY PROTECTION CERTIFICATE
(Instructions and information collection burden statement on reverse)

The following statements are made in accordance with the Privacy Act of 1974 (5 U.S.C. 552a) and the Paperwork Reduction Act (PRA) of 1995.

Application is required in order to determine if a plant variety protection certificate is to be issued (7 U.S.C. 2421). Information is held confidential until certificate is issued (7 U.S.C. 2426).

1. NAME OF OWNER
Kansas Agricultural Experiment Station

2. TEMPORARY DESIGNATION OR EXPERIMENTAL NAME
KS03HW158

3. VARIETY NAME
RonL

4. ADDRESS *(Street and No., or R.F.D. No., City, State, and ZIP Code, and Country)*
Kansas State University
Waters Hall
Manhattan KS 66506

5. TELEPHONE *(include area code)*
785.532.6147

6. FAX *(include area code)*
785.532.6563

FOR OFFICIAL USE ONLY

PVPO NUMBER
#200700244

FILING DATE
March 23, 2007

7. IF THE OWNER NAMED IS NOT A "PERSON", GIVE FORM OF ORGANIZATION *(corporation, partnership, association, etc.)*
University

8. IF INCORPORATED, GIVE STATE OF INCORPORATION

9. DATE OF INCORPORATION

10. NAME AND ADDRESS OF OWNER REPRESENTATIVE(S) TO SERVE IN THIS APPLICATION. *(First person listed will receive all papers)*
T. Joe Martin
KSU Agricultural Research Center-Hays
1232 240th Avenue
Hays KS 67601-9228

FEES RECEIVED

FILING AND EXAMINATION FEES:
$ 4,382.00
DATE 3/23/07

CERTIFICATION FEE:
$ 768.00
DATE 4/27/07

11. TELEPHONE (Include area code)
785.625.3425

12. FAX (Include area code)
785.623-4369

13. E-MAIL
jmartin@ksu.edu

14. CROP KIND *(Common Name)*
Wheat

16. FAMILY NAME *(Botanical)*
Gramineae

15. GENUS AND SPECIES NAME OF CROP
Triticum aestivum

17. IS THE VARIETY A FIRST GENERATION HYBRID?
☐ YES ☒ NO

18. DOES THE VARIETY CONTAIN ANY TRANSGENES? ***(OPTIONAL)***
☐ YES ☒ NO

IF SO, PLEASE GIVE THE ASSIGNED USDA-APHIS REFERENCE NUMBER FOR THE APPROVED PETITION TO DEREGULATE THE GENETICALLY MODIFIED PLANT FOR COMMERICALIZATION. _____

19. CHECK APPROPRIATE BOX FOR EACH ATTACHMENT SUBMITTED (Follow instructions on reverse)

a. ☒ Exhibit A. Origin and Breeding History of the Variety
b. ☒ Exhibit B. Statement of Distinctness
c. ☒ Exhibit C. Objective Description of Variety
d. ☒ Exhibit D. Additional Description of the Variety *(Optional)*
e. ☒ Exhibit E. Statement of the Basis of the Owner's Ownership
f. ☒ Exhibit F. Declaration Regarding Deposit
g. ☒ Voucher Sample *(3,000 viable untreated seeds or, for tuber propagated varieties, verification that tissue culture will be deposited and maintained in an approved public repository)*
g. ☒ Filing and Examination Fee ($4,382), made payable to "Treasurer of the United States" *(Mail to the Plant Variety Protection Office)*

20. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE SOLD AS A CLASS OF CERTIFIED SEED? *(See Section 83(a) of the Plant Variety Protection Act)*
☒ YES *(If "yes", answer items 21 and 22 below)* ☐ NO *(If "no", go to item 23)*

21. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE LIMITED AS TO NUMBER OF CLASSES?
☒ YES ☐ NO
IF YES, WHICH CLASSES? ☒ FOUNDATION ☒ REGISTERED ☒ CERTIFIED

22. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE LIMITED AS TO NUMBER OF GENERATIONS?
☒ YES ☐ NO
IF YES, SPECIFY THE NUMBER 1,2,3, etc. FOR EACH CLASS.
☐ FOUNDATION ☐ REGISTERED ☐ CERTIFIED
(If additional explanation is necessary, please use the space indicated on the reverse.)

23. HAS THE VARIETY (INCLUDING ANY HARVESTED MATERIAL) OR A HYBRID PRODUCED FROM THIS VARIETY BEEN SOLD, DISPOSED OF, TRANSFERRED, OR USED IN THE U. S. OR OTHER COUNTRIES?
☐ YES ☒ NO
IF YES, YOU MUST PROVIDE THE DATE OF FIRST SALE, DISPOSITION, TRANSFER, OR USE FOR EACH COUNTRY AND THE CIRCUMSTANCES. *(Please use space indicated on reverse.)*

24. IS THE VARIETY OR ANY COMPONENT OF THE VARIETY PROTECTED BY INTELLECTUAL PROPERTY RIGHT *(PLANT BREEDER'S RIGHT OR PATENT)*?
☐ YES ☒ NO
IF YES, PLEASE GIVE COUNTRY, DATE OF FILING OR ISSUANCE AND ASSIGNED REFERENCE NUMBER. *(Please use space indicated on reverse.)*

25. The owners declare that a viable sample of basic seed of the variety has been furnished with application and will be replenished upon request in accordance with such regulations as may be applicable, or for a tuber propagated variety a tissue culture will be deposited in a public repository and maintained for the duration of the certificate.

The undersigned owner(s) is(are) the owner of this sexually reproduced or tuber propagated plant variety, and believe(s) that the variety is new, distinct, uniform, and stable as required in Section 42, and is entitled to protection under the provisions of Section 42 of the Plant Variety Protection Act.

Owner(s) is (are) informed that false representation herein can jeopardize protection and result in penalties.

SIGNATURE OF OWNER		SIGNATURE OF OWNER	
Forrest Chumley by RdS			
NAME *(Please print or type)*		NAME *(Please print or type)*	
Dr. Forrest Chumley			
CAPACITY OR TITLE	DATE	CAPACITY OR TITLE	DATE
Assoc Director of Research	3-19-07		

(See reverse for instructions and information collection burden statement)

#200700244

GENERAL INSTRUCTIONS: To be effectively filed with the Plant Variety Protection Office (PVPO), **ALL** of the following items must be **received** in the PVPO: (1) Completed application form signed by the owner; (2) completed exhibits A, B, C, E, F; (3) for a tuber reproduced variety, verification that a viable *(in the sense that it will reproduce an entire plant)* tissue culture will be deposited and maintained in an approved public repository; and (4) payment by credit card or check drawn on a U.S. bank for $4,382 ($518 filing fee and $3,864 examination fee), payable to "Treasurer of the United States" *(See Section 97.6 of the Regulations and Rules of Practice)*. **NEW:** With the application for a seed reproduced variety **or by direct deposit soon after filing**, the applicant must provide at least 3,000 viable untreated seeds of the variety *per se*, and for a hybrid variety at least 3,000 untreated seeds of each line necessary to **reproduce** the variety. Partial applications will be held in the PVPO for not more than 90 days; then returned to the applicant as un-filed. Mail application and other requirements to Plant Variety Protection Office, AMS, USDA, Room 401, NAL Building, 10301 Baltimore Avenue, Beltsville, MD 20705-2351. Retain one copy for your files. All items on the face of the application are self explanatory unless noted below. Corrections on the application form and exhibits must be initialed and dated. **DO NOT** use masking materials to make corrections. If a certificate is allowed, you will be requested to send a payment by credit card or check payable to "Treasurer of the United States" in the amount of $768 for issuance of the certificate. Certificates will be issued to owner, not licensee or agent.

NOTES: It is the responsibility of the applicant/owner to keep the PVPO informed of any changes of address or change of ownership or assignment or owner's representative during the life of the application/certificate. The fees for filing a change of address; owner's representative; ownership or assignment; or any modification of owner's name is specified in Section 97.175 of the regulations. *(See Section 101 of the Act, and Sections 97.130, 97.131, 97.175(h) of the Regulations and Rules of Practice.)*

Plant Variety Protection Office
Telephone: (301) 504-5518 **FAX:** (301) 504-5291
General E-mail: PVPOmail@usda.gov
Homepage: http://www.ams.usda.gov/science/pvpo/PVPindex.htm

SPECIFIC INSTRUCTIONS:

To avoid conflict with other variety names in use, the applicant must check the appropriate recognized authority and **provide evidence** that the permanent name of the application variety (even if it is a parental, inbred line) has been cleared by the appropriate recognized authority before the Certificate of Protection is issued. For example, for agricultural and vegetable crops, contact: U.S. Department of Agriculture, Agricultural Marketing Service, Livestock and Seed Programs, **Seed Regulatory and Testing Branch**, 801 Summit Crossing Place, Suite C, Gastonia, North Carolina 28054-2193 Telephone: (704) 810-8870. http://www.ams.usda.gov/lsg/seed.htm.

ITEM

19a. Give:
(1) the genealogy, including public and commercial varieties, lines, or clones used, and the breeding method;
(2) the details of subsequent stages of selection and multiplication;
(3) evidence of uniformity and stability; and
(4) the type and frequency of variants during reproduction and multiplication and state how these variants may be identified

19b. Give a summary of the variety's distinctness. Clearly state how this application variety may be distinguished from all other varieties in the same crop. If the new variety is most similar to one variety or a group of related varieties:

(1) identify these varieties and state all differences objectively;
(2) attach replicated statistical data for characters expressed numerically and demonstrate that these are clear differences; and
(3) submit, if helpful, seed and plant specimens or photographs (prints) of seed and plant comparisons which clearly indicate distinctness.

19c. Exhibit C forms are available from the PVPO Office for most crops; specify crop kind. Fill in Exhibit C (Objective Description of Variety) form as completely as possible to describe your variety.

19d. Optional additional characteristics and/or photographs. Describe any additional characteristics that cannot be accurately conveyed in Exhibit C. Use comparative varieties as is necessary to reveal more accurately the characteristics that are difficult to describe, such as plant habit, plant color, disease resistance, *etc.*

19e. Section 52(5) of the Act requires applicants to furnish a statement of the basis of the applicant's ownership. An Exhibit E form is available from the PVPO.

20. If "Yes" is specified *(seed of this variety be sold by variety name only, as a class of certified seed)*, the applicant **MAY NOT** reverse this affirmative decision after the variety has been sold and so labeled, the decision published, or the certificate issued. However, if "No" has been specified, the applicant may change the choice. *(See Regulations and Rules of Practice, Section 97.103).*

23. *See Sections 41, 42, and 43 of the Act and Section 97.5 of the regulations for eligibility requirements.*

24. *See Section 55 of the Act for instructions on claiming the benefit of an earlier filing date.*

22. CONTINUED FROM FRONT *(Please provide a statement as to the limitation and sequence of generations that may be certified.)*

Seed will be sold summer of 2007 in US

23. CONTINUED FROM FRONT *(Please provide the date of first sale, disposition, transfer, or use for each country and the circumstances, if the variety (including any harvested material) or a hybrid produced from this variety has been sold, disposed of, transferred, or used in the U.S. or other countries.)*

24. CONTINUED FROM FRONT *(Please give the country, date of filing or issuance, and assigned reference number, if the variety or any component of the variety is protected by intellectual property right (Plant Breeder's Right or Patent).)*

RonL, PVP Application
Exhibit A: Origin and Breeding History of the Variety

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RonL was selected from the cross Trego/CO960293 which was made in the greenhouse at Hays, KS in the spring of 1999.

Parents:
Trego = Kansas developed hard white winter wheat.
CO960293 = A Colorado State developed hard red winter wheat line that was selected from the cross PI222668/TAM107//CO85034.

F_1: Grown in the greenhouse at Hays, KS in the fall of 1999. Seed from all plants bulked at harvest. Segregation was not noted among the F_1 plants.

F_2: Grown in the greenhouse in the spring of 2000. They were mechanically inoculated with wheat streak mosaic virus (WSMV) and WSMV resistant plants were selected. Red seeded plants were discarded.

F_3: Grown in the greenhouse the fall of 2000. Ten plants from each hard white F_2 were inoculated with WSMV and grown to maturity, only WSMV resistant plants that did not have a late maturity were harvested.

F_4: The Selected plant rows were planted in the spring greenhouse in 2001. They were inoculated with WSMV and grown to maturity. Only homozygous WSMV resistant rows were selected that did not have a late maturity. Six head selections were made from each harvested row.

F_5: The bulk seed from the selected rows were tested in preliminary yield tests at two locations in 2002. The head selections were planted as headrows and grown out at Hays KS and one row was harvested after making 6 additional reselections from the row. Segregation was not observed within the line. Selection criteria included grain yield, test weight, height, maturity, resistance to shattering, leaf rust resistance, sprouting tolerance, polyphenol oxidase level, dough mixing strength as measured with a mixograph, and white pan bread baking characteristics.

F_6: In 2003 the bulk was tested in 3 western Kansas preliminary yield tests. The bulk was also grown in a 6 by 30 ft pure seed increase plot at Hays which provided seed for testing in 2004. No segregation was noted in the line. The head selections were grown as head rows at Hays and a single row was selected from which 6 head selections were made. . Selection criteria included grain yield, test weight, height, maturity, resistance to shattering, resistance to WSMV, leaf rust resistance, Stripe rust resistance, sprouting tolerance, polyphenol oxidase level, dough mixing strength as measured with a mixograph, and white pan bread baking characteristics.

F_7: Bulk seed from the 2004 pure seed increase plot was used to enter KS03HW158 in replicated yield tests known as the Kansas Intra-State Nursery (KIN). This nursery is a

replicated yield test planted at 18 Kansas locations. Two pure seed increase plots were grown at Hays in 2004 with seed produced on the selected headrow. No segregation was observed in the line. Selection criteria included grain yield, test weight from the 9 locations in western Kansas, height, maturity, resistance to shattering, leaf rust resistance, sprouting tolerance, polyphenol oxidase level, dough mixing strength as measured with a mixograph, and white pan bread baking characteristics.

F_8: In 2005 KS03HW158 was tested in the KIN, the Southern Regional Performance Nursery and the Kansas Performance Tests With Winter Wheat Varieties. Seed produced in the pure seed increase plots was used to produce a small increase for the variety. No segregation was noted in the increase.

F_9: In 2006 an additional increase was planted at Hays. Segregation was not detected in this grow out. The variety was again tested in all the same nurseries as it was tested in 2005.

F_{10} : In 2007 seed of RonL (KS03HW158) will be produced and offered for sale to the public for the first time as class of Certified seed. Segregation has not been noted by the time application was filed.

RonL is uniform. Variants are limited to: slightly taller plants that occur at a frequency of less than 1 in 1,000 plants, plants with brown glumes that occur at a frequency of less than 1 in 1,000 plants, and plants that produce seed with a red seed coat that occur at a frequency of less than 1 in 200 plants. The variants in RonL as well as the typical plants in RonL are commercially acceptable.

RonL is stable. When sexually reproduced, the variety remains unchanged in its essential and distinctive characteristics. RonL was observed to be uniform and stable during the last 4 generations.

RonL PVP Application
Exhibit B: Statement of Distinctness

#200700244

RonL is most similar to Trego.

RonL is different than Trego because RonL has the wheat streak mosaic virus resistance derived from CO960293 (1 & 2). Replicated yield and volume weight field data are presented in Table 1.

1. Haley et al 2002. Registration of CO960293-2 wheat germplasm resistant to wheat streak mosaic virus and Russian wheat aphid. Crop Sci. 42:1381-1382.
2. Seifers et al 2006. Temperature sensitivity and efficacy of wheat streak mosaic virus resistance derived from CO960293 wheat. Plant Disease 90:623-628.

Table 1. Grain yield and volume weight of wheat cultivars either healthy (H) or infected (I) by *Wheat streak mosaic virus* over 2 years[Z] at Hays, KS

		Yield (Kg/ha)		Volume weight (Kg/m^3)	
		Year		Year	
Cultivar	Treatment	2005	2006	2005	2006
RonL	H	6,114A	2,249ABC	803AB	756A
RonL	I	5,937AB	2,265AB	807A	757A
Trego	H	5,476BCD	2,178ABC	796BCD	748A
Trego	I	4,077F	1,511D	740F	709BC
Jagger	H	5,271CD	2,405A	772E	698D
Jagger	I	4,236F	1,970C	728G	642G
2145	H	5,014DE	1,997BC	797CDE	706CD
2145	I	2,726G	1,547D	704H	666F
CV		7.9	9.5	0.8	0.9

[Z]Treatments not having the same letter in common are significantly different using the least significance difference test (P=0.05).

Temperature Sensitivity and Efficacy of *Wheat streak mosaic virus* Resistance Derived from CO960293 Wheat

D. L. Seifers, Professor, and **T. J. Martin,** Professor, Kansas State University Agricultural Research Center-Hays, Hays 67601; **T. L. Harvey,** Professor, Department of Entomology, Kansas State University, Manhattan 66506; **S. Haber,** Cereal Research Center, Agriculture & Agri-Food Canada, Winnipeg, Canada; and **S. D. Haley,** Soil and Crop Science Department, Colorado State University, Fort Collins 80523

ABSTRACT

Seifers, D. L., Martin, T. J., Harvey, T. L., Haber, S., and Haley, S. D. 2006. Temperature sensitivity and efficacy of *Wheat streak mosaic virus* resistance derived from CO960293 wheat. Plant Dis. 90:623-628.

Wheat yields often are limited by infection by *Wheat streak mosaic virus* (WSMV). Host plant resistance to WSMV can reduce losses. This study was conducted to characterize a new source of temperature-sensitive resistance found in CO960293 wheat. The source of the temperature-sensitive resistance in CO960293 is unknown. Parental and other wheat lines were tested for WSMV resistance using 51 WSMV isolates under different temperatures to determine the stability of the resistance, and yield trials were conducted in the field for 3 years. All parental wheat lines became infected by WSMV at all temperatures and were infective in back assay to 'Tomahawk' wheat. No WSMV isolate defeated the resistance of CO960293 at 18°C. Yield of CO960293 in field trials was reduced in only 1 of 3 years. Our data demonstrate that this wheat line can be a valuable source of resistance to WSMV in wheat programs, particularly in areas where temperatures are cool following planting in the fall.

Infection of wheat by *Wheat streak mosaic virus* (WSMV), vectored by the wheat curl mite (WCM) *Aceria tosichella* Keifer (15), causes wheat streak mosaic, a serious disease of wheat in Kansas causing losses as high as 13% reduction in yield of the wheat crop (5,14,18).

High levels of WSMV resistance are not available in commercial cultivars. Lower levels of resistance are available, but these cultivars suffer significant yield losses under high WSMV pressure (12). The KS96HW10-3 germ plasm derived from *Agropyron intermedium* has been shown to have a high level of resistance under field conditions (13). The resistance from *A. intermedium* has been shown to be tightly linked to the gene identified as *wsm1*, and its presence can be verified using the J15 (SCAR) DNA marker (17). This source was demonstrated to be temperature-sensitive; however, under field conditions, this resistance still was effective in preventing losses to WSMV infection (13). To date, this is the only temperature-sensitive source of resistance to WSMV that has been identified.

In 1999, wheat from the Hard Winter Wheat Regional Germplasm Observation Nursery was tested at Hays, KS under natural infestation with WCM viruliferous for WSMV in a WSMV resistance screening nursery (8). All plants of wheat line CO960293 (PI222668/TAM 107// CO850034 pedigree) remained symptomless in this field test. The symptomless plants tested negative in enzyme-linked immunosorbent assay (ELISA) against antisera to WSMV and the High Plains virus. A homozygous single-plant selection made from CO960293 has been released as germ plasm CO960293 (3).

The objective of this work was to characterize the type and effectiveness of the WSMV resistance of CO960293 and other wheat germ plasm under greenhouse, growth-chamber, and field conditions.

MATERIALS AND METHODS

Sources and maintenance of WSMV isolates. Fifty-one WSMV isolates were used in these studies (Table 1). The susceptible wheat cv. Tomahawk was mechanically inoculated on the first leaf using the finger-rub technique (11) and held in a growth chamber at 20 to 22°C for 2 weeks. The symptomatic plant tissue then was frozen at –80°C until needed. Inoculations in all experiments were done using thawed portions of such frozen tissue. Inoculum was prepared by grinding infected tissue in a mortar and pestle at a 1:10 (wt/vol) dilution in 0.02 M potassium phosphate buffer (pH 7). The homogenate was filtered through cheesecloth, and 1 g of abrasive (Crystolon flour B, 600 mesh; Norton Co., Worcester, MA) was added for 100 ml of inoculum. This method of inoculum preparation and inoculation used throughout this investigation has been described previously (11).

Infectivity assays. Tomahawk wheat was mechanically inoculated as described above at the single-leaf stage with 1:5 (wt/vol) extracts prepared from different wheat lines. Inoculated plants were held in a greenhouse under natural lighting and rated for numbers of symptomatic plants at 14 days post inoculation (DPI).

Indirect ELISA. Leaf tissue was ground 1:30 (wt/vol) in 0.05 M carbonate buffer, pH 9.6 (1). Extracts (200 µl) were placed in wells of ELISA plates (Immulon 1; Dynatech Laboratories, Inc., Chantilly, VA) for 1 h at 37°C. Following rinsing, the wells were incubated for 1 h at 37°C with a WSMV antivirus antibody (12) dilution of 5 µg/ml in blocking buffer (5% nonfat dry milk, 0.01% antifoam A, and 0.02% sodium azide in phosphate-buffered saline, pH 7.4). The plates then were rinsed and blocked for 1 h at 37°C. Then, 200 µl of anti-rabbit antibody/alkaline phosphatase conjugate (Southern Biotechnology Associates, Birmingham, AL) in blocking buffer (1:3,000 vol/vol) was added per well. The plates were held at 37° C for 1 h. The plates then were rinsed, and 200 µl of substrate (p-nitrophenyl phosphate, 0.714 mg/ml) in substrate buffer (1) was added to each well. The plates then were held at room temperature (20 to 22°C) for 30 min. Absorbance was measured at 405 nm using a Titertek Multiscan plate reader (Flow Laboratories, Inc., McLean, VA). Absorbance values were considered positive if they were twice those of the equivalent mock-inoculated control.

WSMV testing of wheat lines. Seed of wheat lines CO960255, CO960223, CO960293, CO960315, CI6501, KS96H10-3, plant introduction (PI)222651, PI222655, PI222661, PI222666, PI222668, PI222671, PI222679, PI222680, PI222681, and PI222682 were planted into 30-by-50-cm soil-filled metal flats at 10 seed per row for each entry with eight rows per flat for a total of four flats (two for each temperature). Plants were mechanically inoculated as described above with WSMV isolate

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Contribution No. 05-292-J from the Kansas Agricultural Experiment Station, Manhattan, Kansas 66506. Research was supported in part by a grant from the Kansas Wheat Commission.

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Sidney 81 at the single-leaf stage. One set of plants was held at 18°C in a growth chamber for 14 days with 8 h of illumination (250 μE sec^{-1} m^{-2}) per day and one set of plants was held for 14 days at 24°C in a growth chamber with the same light conditions. Symptom expression of both sets was recorded at 14 DPI. The experiment was conducted twice. All plantings in these and other experiments conducted in the course of this investigation used a Harney clay loam (fine montmorillonitic, mesic Typic Argiustoll) soil. Lines PI222651 to PI222682 were tested to determine whether they might have resistance to WSMV because preliminary tests showed that the parental lines PI222668, TAM 107, and CO850034 were susceptible to infection by WSMV (D. L. Seifers, *unpublished*).

WSMV infectivity testing of CO960293 and parental wheat lines. Wheat lines CO960293, CO850034, PI222668, and TAM107 were planted into 30-by-50-cm soil-filled (soil type as described above) metal flats at 10 seed per row for each entry so that each flat contained one row for each wheat line. Two sets of this planting were done so that plants were available for the 18 and 24°C temperature treatments. The plants were held at 18°C until they were at the single-leaf stage and then were mechanically inoculated as described above on the first leaf with a 1:10 wt/vol dilution of the Sidney 81 isolate of WSMV. The plants then were placed immediately in growth chambers at either the 18 or 24°C temperature treatment. A randomized complete block design was used with the location of wheat lines within flats randomized, as were the positions of the flats within the growth chambers. At 21 DPI, all symptomatic tissues of the third leaves were bulked (within a wheat line) and ground in 0.02 M KPO_4, pH 7, buffer at a 1:25 wt/vol dilution. From this preparation, serial dilutions of 1:50, 1:100, 1:200, 1:400, 1:800, 1:1,600, and 1:3,200 were prepared. The extracts were used to inoculate Tomahawk wheat plants (7 to 10 plants per dilution at the single-leaf stage) as described above. The inoculated Tomahawk infectivity assay plants were held in a greenhouse under natural lighting (temperatures ranged from 19 to 28°C) prior to determining the number of symptomatic plants at 14 DPI. The experiment was conducted three times. Infection percentage data were transformed with the arcsine transformation before analysis of variance because the range of percentages among treatments was greater than 40 (6); untransformed data will be presented. The analysis of variance (ANOVA) for the data was conducted using SAS (version 8; SAS Institute, Cary, NC) and significant treatment effects were determined using the least significance difference (LSD) test at $P = 0.05$.

***Wsm1* gene probe.** Healthy wheat leaves of CO960293, CO960315, CO960255, CI15092, KS96HW10-3, TAM 107, and the Canadian spring wheat cvs. AC Elsa and AC Superb were tested for the presence of the J15 (SCAR) DNA marker, which has been shown to be tightly linked to the presence of the resistance gene, *wsm1*, derived from *Thinopyrum intermedium* (17).

DNA extraction. Wheat leaf pieces (approximately 3 by 1 cm each) were placed in individual 15-ml tubes and lyophilized overnight. The tissue in each tube was pulverized by vigorous abrasion with acid-washed sand (2 ml of sand per tube, 5 min of agitation on a commercial paint shaker), then extracted with 6 ml of DNA extraction buffer (0.1 M Tris-HCl, pH 8.7; 0.05 M EDTA, pH 8.0; 1.5 M NaCl; and 1% cetyltrimethylammonium bromide) to which 30 μl of proteinase K (10 mg/ml in Tris-EDTA buffer [10 mM Tris-HCl, pH 7.6, and 1 mM EDTA] pH 7.6) and 660 μl of 20% (wt/vol) sodium dodecyl sulfate were added (2 h of incubation at 65°C in a water bath), followed by addition in a fume hood of 6 ml of chloroform/isoamyl alcohol (24:1). After slow shaking for 30 min, the tubes were centrifuged at 5,000 × *g* for 10 min. The supernatant (approximately 3 ml) from each tube was removed to a fresh tube and 3 ml of ice-cold isopropanol was added. The DNA which precipitated at the interface between the two phases was removed (1 ml) to a microcentrifuge tube, then pelleted by centrifugation at 15,000 × *g* for 20 min. After washing with 70% (vol/vol) ethanol, DNA

Table 1. *Wheat streak mosaic virus* (WSMV) isolate designation, origin, isolation host, and year of isolation

WSMV isolate	Location of origin	Isolation host	Year isolated
BT95	Kansas	Wheat	1995
CK93	Kansas	Wheat	1993
CL93	Kansas	Wheat	1993
CM93	Kansas	Wheat	1993
CO85	Kansas	Wheat	1985
CO87	Colorado	Wheat	1987
El Batán 3	Mexico	Wheat	1996
EW95	Kansas	Wheat	1995
FO93	Kansas	Wheat	1993
GH95	Kansas	Wheat	1995
GO93	Kansas	Wheat	1993
GY93	Kansas	Wheat	1993
H81	Kansas	Wheat	1981
H88	Kansas	Wheat	1988
H94PM	Kansas	Wheat	1994
H94S	Kansas	Wheat	1994
H94USDA	Kansas	Wheat	1994
H95LB	Kansas	Wheat	1995
H95S	Kansas	Wheat	1995
H98	Kansas	Wheat	1998
HM93	Kansas	Wheat	1993
HV91	Kansas	Wheat	1991
ID96	Idaho	Wheat	1996
ID99	Idaho	Maize	1999
IHC	Canada	Wheat	1989
KM93	Kansas	Wheat	1993
KY00	Kentucky	Wheat	2000
KY0083SV	Kentucky	Wheat	2000
LC95	Kansas	Wheat	1995
LG92	Kansas	Wheat	1992
MO00	Missouri	Wheat	2000
MO99	Missouri	Wheat	1999
MON96	Montana	Wheat	1996
ND	North Dakota	Wheat	1969
NE96	Nebraska	Wheat	1996
OK98	Oklahoma	Wheat	1998
OSU	Unknown	Unknown	Unknown
PL95	Kansas	Wheat	1995
PN95	Kansas	Wheat	1995
PV106	Ohio	Maize	1964
PV106JM	Ohio	Maize	1964
PV57	Kansas	Wheat	1932
PV91	Kansas	Wheat	1949
RO95	Kansas	Wheat	1995
SD96	South Dakota	Wheat	1996
Sidney 81	Nebraska	Wheat	1981
TK1	Turkey	Wheat	1999
TK2	Turkey	Wheat	1999
TX96	Texas	Wheat	1996
WA99	Washington	Maize	1999
WO93	Ohio	Maize	1993

pellets were left in 70% ethanol overnight at –20°C, centrifuged at 15,000 × *g* for 20 min, and the ethanol decanted. The pellets were allowed to air dry; then, each pellet was dissolved in 250 µl of autoclaved, deionized water.

Amplification of marker DNA by polymerase chain reaction. Amplifications of the 241-bp J15 marker DNA sequence were carried out using a polymerase chain reaction (PCR) DNA amplification kit (cat. no. N808-0130; Perkin Elmer—Roche Diagnostics, Nutley, NJ) and the custom primers left primer (5′–3′) GTA GCA GGG GAA GCT GAA GA and right primer (5′–3′) CCG AGC TCA CAC GCT AAT TT (10/µl). From the kit, the following reagents were added to a microcentrifuge tube: 32 µl of deionized water, 7.5 µl of reverse-transcription PCR buffer, 5 µl of $MgCl_2$, and 5 µl of 10× dNTPs. To this mixture, 2.5 µl of each of the left and right primer DNA, 1 µl of the target DNA sample, and 0.75 µl of Taq DNA polymerase (5 units/µl) were added. After mixing the reagents by gently flicking each microcentrifuge tube, thermal cycling was carried out in a PTC 100-MJ thermocycler (MJ Research, Watertown, MA) for 3 min at 94°C, followed by 34 cycles of 1 min at 94°C, 1 min at 60°C, and 2 min. at 72°C; a 10-min period of annealing at 72°C; and concluding with cooling overnight at 4°C.

PCR DNA products were analyzed in a 1.5% agarose gel containing ethidium bromide (2). The PCR product (10 µl) was mixed with 1.5 µl of PCR Loading Buffer (Perkin Elmer—Roche Diagnostics) and loaded into the gel, and electrophoresis was conducted for 2 h at 40 V. The presence or absence of the J15 SCAR DNA marker was noted compared with the 241-bp single band produced by the PCR reaction with DNA extracted from the positive control wheat lines CI15092 and KS96H10-3.

Temperature and WSMV isolate interactions for CO960293 resistance. Seed of CO960293 wheat was sieved to uniform size and planted in 30-by-50-cm soil-filled (11 kg of soil) metal flats, each with 22 rows (12 cm long). Six flats were used in each of three runs of the experiment (three flats for the 18°C treatment and three flats for the 24°C treatment). When the plants were at the single-leaf stage, each row was thinned to 10 plants of uniform size. The plants in a row then were mechanically inoculated (as described above) with one of the 51 WSMV isolates. One row in each flat was mock inoculated with buffer and abrasive as the healthy control. A randomized complete block design was used and the location of WSMV isolates and the healthy wheat control within flats was randomized, as were the locations of the flats in the growth chambers. The outer two rows of each flat were planted with wheat that was not inoculated so that all inoculated plants were surrounded by wheat on each side. Plants were held in a growth chamber at either 18 or 24°C. Plants were rated on a daily basis for first-symptom expression and for severity (whole plant and averaged for all plants in a row) at 21 DPI according to the following symptom scale: 1 = no symptoms, 2 = faint mosaic, 3 = moderate mosaic, 4 = severe mosaic, and 5 = severe mosaic with yellowing of leaves. At 21 DPI, the plants within a row were cut at the soil surface, bulked for a given WSMV isolate or healthy control, and placed in labeled paper bags, and the plant tissue was dried to a constant weight.

ANOVAs (first-symptom expression, symptom severity, and dry weight) were performed using the general linear models procedure of SAS. In the analysis, the uninoculated, symptomless controls and plants inoculated with the El Batán 3 WSMV isolate were assigned a 15-day value to facilitate data analysis. Treatment means were separated using the Tukey's Studentized Range test.

Confirmation of infectivity of selected WSMV isolates on CO960293 grown at 18 and 24°C. The WSMV isolates LG92, CM93, El Batán 3, ND, WO93, and IHC were used to mechanically inoculate CO960293 and Tomahawk wheat. Wheat was planted in two 30-by-50-cm soil-filled metal flats each flat with six rows. Each row of plants was inoculated (as described above) at the single-leaf stage and then one of the two flats was placed at 18°C and the other at 24°C. After 2 weeks at 24°C, the third leaf was harvested from each plant and bulked within a row inoculated with a given WSMV isolate and a 1:5 wt/vol dilution of extract was prepared (as described above). The extract from plants infected with each virus isolate was used to inoculate a separate row of Tomahawk wheat seedlings (10 plants) planted 7 days previously. After 3 weeks at 18°C, the third leaves of plants inoculated with a respective WSMV isolate were bulked and used to make a 1:5 wt/vol dilution of extract. This extract was used to mechanically inoculate Tomahawk wheat seedlings planted 1 week previously, as described for the 24°C treatment. The experiment was conducted three times. The WSMV isolates were chosen from among the 51 isolates because of varied reaction for CO960293 wheat observed in the 18 and 24°C isolate interaction study.

Yield trials. Yield trials were conducted to compare the effectiveness of the resistance of CO960293 to that of KS96HW10-3 that carries resistance from *A. intermedium* (13). Several wheat cultivars with

Table 2. Numbers of symptomatic wheat plants from different lines following mechanical inoculation with *Wheat streak mosaic virus* (WSMV) and being held at 18 or 24°C for 2 weeks following inoculation

	Temperature (°C)[z]	
Wheat line	**18**	**24**
CO960255	8/8	8/8
CO960293	0/10	9/10
CO960315	10/10	10/10
CO960223	15/16	16/16
CI6501	15/15	12/12
KS96HW10-3	0/10	9/9
PI222651	4/4	NT
PI222655	7/7	NT
PI222661	6/6	NT
PI222666	9/9	NT
PI222668	14/14	NT
PI222671	10/10	NT
PI222679	4/4	NT
PI222680	5/5	NT
PI222681	5/5	NT
PI222682	4/4	NT

[z] Numerator represents the total number of symptomatic plants and the denominator the total number of plants mechanically inoculated with WSMV in two experiments; NT = not tested.

Table 3. Percentages of 'Tomahawk' wheat plants infected by *Wheat streak mosaic virus* (WSMV) following mechanical inoculation with different dilutions of plant extract prepared from plants of different wheat lines grown at either 18 or 24°C[z]

	Reciprocal of plant dilution						
Wheat, temperature (°C)	**50**	**100**	**200**	**400**	**800**	**1,600**	**3,200**
CO850034, 18	100 A	83 A-D	56 F-J	38 I-M	13 L-P	60 Q	0 Q
CO850034, 24	100 A	100 A	97 B	93 A-C	83 A-D	59 E-I	42 H-M
CO960293, 18	0 Q	0 Q	0 Q	0 Q	0 Q	0 Q	0 Q
CO960293, 24	100 A	100 A	93 A-C	81 B-D	60 E-I	23 K-O	7 N-Q
PI222668, 18	100 A	100 A	100 A	87 A-D	47 G-K	23 K-O	7 N-Q
PI222668, 24	100 A	100 A	100 A	87 A-C	73 B-F	42 H-M	20 L-P
TAM 107, 18	100 A	63 D-H	45 G-K	8 N-Q	0 Q	0 Q	0 Q
TAM 107, 24	100 A	93 A-C	87 A-D	80 B-E	70 C-G	40 H-M	17 M-Q

[z] Percentages for the 1:25 dilution are identical to that of the 1:50 dilution and are not shown. Treatments not having a letter in common are significantly different using the least significance difference test ($P = 0.05$).

varying levels of susceptibility to WSMV infection were included: Trego, Jagger, 2145, Ike, Karl 92, and 2137. A randomized complete block experimental design was used for each of 3 years, with each treatment replicated four times. Plots (healthy or infected for each cultivar) consisted of three rows, 2.9 m long with a 0.3-m row spacing, seeded at 50 kg/ha. Planting was done on 22 September 2000, 12 September 2002, and 25 September 2003. Plants were inoculated with the Sidney 81 isolate at the two-to-three leaf stage (13 October 2000, 26 September 2002, and 21 October 2003) using an air-blast inoculation technique (7). Harvest dates were 17 June 2000, 19 June 2002, and 28 June 2003. The ANOVA was conducted using SAS and significant treatment effects were determined using the LSD test at $P = 0.05$.

Table 4. Days to first symptom expression (FSE), symptom severity, and dry weight, of CO960293 wheat mechanically inoculated with different *Wheat streak mosaic virus* (WSMV) isolates and held at 24°C for 3 weeks[w]

Virus isolate or control	FSE[x]	Symptom severity[y]	Dry weight (mg)[z]
Uninoculated control 1	15.0 A	1.0 D	594 AB
Uninoculated control 2	15.0 A	1.0 D	643 AB
Uninoculated control 3	15.0 A	1.0 D	659 AB
El Batán 3	15.0 A	1.0 D	642 AB
WO93	11.2 B	2.2 CD	688 A
CO87	9.7 BC	2.2 CD	611 AB
PV106	9.2 BCD	2.2 CD	582 AB
KY00	9.0 BCD	2.5 BCD	663 AB
RO95	8.5 BCD	3.5 ABC	479 AB
IHC	8.5 BCD	3.0 ABC	530 AB
OSU	8.5 BCD	2.7 BC	550 AB
GO93	8.2 BCD	3.0 ABC	525 AB
H95LB	8.0 CD	2.7 BC	616 AB
H88	8.0 CD	3.0 ABC	574 AB
PV57	8.0 CD	2.7 BC	577 AB
ID96	8.0 CD	2.5 BCD	591 AB
Sidney 81	7.7 CD	3.0 ABC	625 AB
TK1	7.7 CD	3.0 ABC	469 AB
ID99	7.7 CD	3.0 ABC	606 AB
TX96	7.7 CD	3.2 ABC	519 AB
MON96	7.7 CD	3.7 ABC	515 AB
WA99	7.7 CD	3.5 ABC	525 AB
ND	7.7 CD	3.5 ABC	450 AB
PV91	7.5 CD	3.2 ABC	575 AB
CK93	7.5 CD	3.0 ABC	560 AB
MO00	7.2 CD	3.5 ABC	511 AB
BT95	7.2 CD	3.2 ABC	580 AB
CL93	7.2 CD	3.7 ABC	573 AB
HV91	7.2 CD	3.5 ABC	495 AB
GH95	7.2 CD	3.0 ABC	523 AB
SD96	7.2 CD	3.5 ABC	503 AB
EW95	7.2 CD	3.5 ABC	623 AB
GY93	7.2 CD	3.5 ABC	504 AB
H98	7.0 CD	3.0 ABC	568 AB
H81	7.0 CD	2.7 BC	591 AB
KY0083SV	7.0 CD	2.7 BC	629 AB
PN95	7.0 CD	3.5 ABC	480 AB
LG92	7.0 CD	4.5 A	549 AB
PV106JM	7.0 CD	3.5 ABC	548 AB
H95S	6.7 CD	3.5 ABC	497 AB
KM93	6.7 CD	3.2 ABC	575 AB
OK98	6.7 CD	3.2 ABC	611 AB
TK2	6.7 CD	3.0 ABC	570 AB
CM93	6.7 CD	4.0 AB	419 B
H94PM	6.7 CD	3.5 ABC	530 AB
HM93	6.7 CD	3.7 ABC	552 AB
FO93	6.7 CD	3.7 ABC	558 AB
PL95	6.7 CD	3.2 ABC	566 AB
LC95	6.7 CD	3.0 ABC	546 AB
NE96	6.5 D	3.0 ABC	562 AB
H94USDA	6.5 D	3.2 ABC	515 AB
H94S	6.5 D	3.5 ABC	524 AB
CO85	6.5 D	3.2 ABC	537 AB
MO99	6.5 D	3.2 ABC	561 AB

[w] Treatments not having a letter in common are significantly different using the Tukey's Studentized Range test ($P = 0.05$).

[x] FSE values represent average days post inoculation for appearance of first symptoms for a given treatment averaged over the three experiments.

[y] Symptom severity ratings: 1 = no symptoms, 2 = faint mosaic, 3 = moderate mosaic, 4 = severe mosaic, and 5 = severe mosaic with yellowing of leaves. The ratings were based on the top three leaves collectively of all plants in a row for each treatment for each experiment.

[z] Dry weight values represent average value from three experiments. The dry weight of all plants in a row for a given treatment was combined in each experiment.

RESULTS

WSMV testing of wheat lines. Plants of all wheat lines except CO960293 and KS96HW10-3 developed mosaic symptoms when held at 18°C (Table 2). All lines tested at 24°C developed mosaic symptoms, including CO960293 and KS96HW10-3. Symptomatic tissue was positive in ELISA, and the symptomless tissue of CO960293 wheat and the KS96HW10-3 control held at 18°C all were negative in ELISA (*data not shown*).

WSMV infectivity assay of CO960293 and parental wheat lines. The parent lines of CO960293 (CO850034, PI 222668, and TAM 107), all became symptomatic and the tissue was infective for plants grown at both 18 and 24°C (Table 3). At 18°C, only CO960293 was not infective to Tomahawk wheat plants, although it was infective to Tomahawk assay plants when held at 24°C. TAM 107, held at 18°C, was infective only to the 1:400 dilution. At the 1:800 dilution, significant differences in percentages of infected Tomahawk wheat plants were observed between the 18 and 24°C treatments for each wheat line. We also tested wheat lines of similar origin to PI222668 (PI22651 to PI222682) and these also developed symptoms at 18°C (Table 2).

***Wsm1* gene probe.** The chromosome 4A substitution line CI15092 and the chromosome 4-DL translocation line KS96HW10-3 contained the identical 241-bp SCAR marker (*data not shown*). By contrast, no SCAR band was detected in PCR amplifications of DNA extracts of CO960293, CO960315, CO960255, TAM 107, or the spring wheat cvs. AC Elsa or AC Superb.

Temperature and WSMV isolate interactions on CO960293 resistance. At 18°C, none of the 51 WSMV isolates caused symptom expression in CO960293, nor did they cause any significant reduction in dry weight when compared with healthy controls (*data not shown*). However, at 24°C, all WSMV isolates except El Batán 3 induced symptoms (Table 4). First-symptom expression on CO960293 varied from 6.7 to 11.2 days and was significantly different for some isolates. All uninoculated controls and plants inoculated with the El Batán 3 isolate were symptomless; however, they were assigned a 15-day value to facilitate data analysis. Symptom severity varied among isolates and ranged from 2.2 to 4.5. The WO93 and the CM93 WSMV isolates were significantly different from each other for dry weight, whereas the other isolates were not different.

Confirmation of infectivity of selected WSMV isolates on CO960293 grown at 18 and 24°C. At 24°C, all WSMV isolates caused symptoms in Tomahawk wheat and extracts from these plants were infective to all Tomahawk wheat assay plants in all three experiments. However, at 24°C, all isolates except El Batán 3 caused symptoms in CO960293 and extracts from the symptomatic leaves were infective to Tomahawk assay plants. In contrast, extracts prepared from the symptomless leaves of CO960293 wheat inoculated with the El Batán 3 isolate were not infective to Tomahawk wheat. At 18°C, none of the CO960293 wheat plants inoculated with the six WSMV isolates developed symptoms, nor were extracts from these plants infective to Tomahawk wheat. In contrast, all WSMV isolates except El Batán 3 caused symptoms to develop in Tomahawk wheat held at 18°C and extracts from the symptomatic Tomahawk wheat was infective to all Tomahawk assay plants in all experiments. Extracts from the symptomless Tomahawk wheat inoculated with the El Batán 3 isolate held at 18°C were not infective to Tomahawk wheat.

Yield trials. In both 2000 and 2003, the grain yield and volume weight of entries inoculated with the Sidney 81 isolate of WSMV were not significantly different from healthy controls for both CO960293 and KS96H W10-3 (Table 5). For all other wheat lines, grain yield and volume weight of the inoculated treatment was significantly lower than its healthy control, except for the volume weight of Jagger in 2003. In 2002, all infected treatments except KS96HW10-3 were significantly lower in yield than their healthy controls.

DISCUSSION

Wheat line CO960293 was grown in the field and was infested naturally by WCM viruliferous for WSMV (8). In this setting, it was observed that none of the plants of this line were symptomatic, whereas all plants (CO960255 and CO960315) in rows growing adjacent to it on either side were symptomatic. When plants grown from seed from these CO960293 plants were tested in replicated experiments mechanically inoculated with WSMV at 18 and 24°C, only the plants arising from seed of the symptomless CO96293 plants were symptomless at 18°C (Table 2). This confirmed that the symptomless plants in the field were not escapes and that CO960293 had temperature-sensitive resistance to WSMV as observed for KS96HW10-3 (13).

CO960293 was selected from the cross PI222668/TAM 107//CO850034. Thus, our assumption was that one of these lines was the source of the resistance seen in the CO960293 plants. When we tested these at 18 and 24°C, we found that all parents developed symptoms and were highly infective in back assay at 18°C. The observation that TAM 107 was infected was not unexpected because this wheat line has been demonstrated to be susceptible to mechanical inoculation by WSMV (13). TAM 107 was resistant to WCM infestation (10,19), but we have demonstrated that this resistance is not effective against new biotypes of WCM (4). No information is available in the literature about either CO850034 or PI22668 being resistant to WSMV. Because many PI sources are heterogeneous, it is possible that the WSMV resistance in CO960293 originated from a rare plant within PI22668 that was used in the original cross. We have evaluated many plants from several different samples of PI222668 and have failed to identify any plants with WSMV resistance. We have observed that the resistance from crosses with CO960293 has been recovered easily, although no extensive data are available concerning genetic control of the temperature sensitive resistance derived from CO960293.

Temperature-sensitive WSMV resistance has been identified in KS96HW10-3 wheat (13). Because this line was grown in both breeding nurseries and greenhouses in both Colorado and Kansas, we explored the possibility that an outcross might have occurred and that CO960293 carries the resistance from *A. intermedium* (13). However, when tissue from CO960293 was tested for the presence of J15 (SCAR) DNA marker, no SCAR band was detected in PCR amplifications of DNA extracts of this line, CO960315, CO960255, TAM 107, or the spring wheat cvs. AC Elsa and AC Superb. By contrast, the chromosome 4A substitution line CI15092 and the chromosome 4-DL translocation line KS96HW10-3 (13) contained the identical 341-bp SCAR marker shown to be tightly linked to the *Wsm1* gene on chromatin derived from *A. intermedium* chromosome 4 (17). These observations suggest that CO960293 does not carry the resistance from *A. intermedium*.

When CO960293 was tested against 51 different WSMV isolates at 18°C, no plants developed symptoms, nor were any differences in dry weight noted among virus treatments at this temperature. Thus, the resistance present at 18°C was effective regardless of WSMV isolate source. However, at 24°C, all isolates except El Batán 3 incited symptoms. The El Batán 3 WSMV isolate was isolated in Mexico (9) and has a coat protein nucleic acid sequence that varies from the Sidney 81 isolate of WSMV by 20% (16). Such variation may be involved in the apparent lack of systemic infection of CO960293 we observed at 24°C.

In infectivity assays, we showed that the extracts prepared from symptomless plants of CO960293 inoculated by the El Batán 3 isolate and held at either 18 or 24°C were

Table 5. Grain yield and volume weight of wheat cultivars either healthy (H) or infected (I) by *Wheat streak mosaic virus* over 3 years at Hays, KS[y]

		Yield (kg/ha) in year			Volume (kg/m^3) in year		
Cultivar[z]	Treatment	2000	2002	2003	2000	2002	2003
CO960293	H	4,044	3,138	4,791	755	699	753
CO960293	I	4,172	2,392*	4,576	750	688	742
KS96HW10-3	H	4,051	2,573	3,769	795	754	790
KS96HW10-3	I	4,072	2,580	3,581	798	750	784
Trego	H	4,240	3,984	5,711	800	754	804
Trego	I	3,285*	2,761*	3,312*	742*	715*	696*
Jagger	H	3,036	2,633	5,597	772	712	775
Jagger	I	2,452*	1,236*	3,574*	709*	661*	665
2145	H	3,072	2,714	5,415	780	723	795
2145	I	2,035*	490*	1,841*	708*	668*	631*
Ike	H	3,547	2,909	5,308	777	712	790
Ike	I	2,465*	430*	2,997*	703*	654*	741*
Karl 92	H	3,487	2,250	5,006	784	721	800
Karl 92	I	2,606*	1,713*	2,385*	723*	695*	669*
2137	H	3,561	2,520	4,891	771	692	785
2137	I	2,885*	1,145*	3,003*	712*	651*	694*
CV[z]	...	9.7	14.5	9.7	2.0	1.7	2.0
LSD (0.05)	...	571	443	571	22	17	22

[y] Asterisk (*) indicates that this value is significantly different from the healthy control for a given wheat line.
[z] CV = coefficient of variation and LSD = least significant difference.

not infective. However, Tomahawk wheat developed symptoms inoculated with this isolate when held at 24°C and such tissue was infective to assay plants. This indicated that the inoculum was infective and that the lack of symptoms and infectivity in CO960293 at both temperatures was not because of poor-quality inoculum. The lack of symptoms and infectivity observed for CO960293 wheat at both temperatures and Tomahawk wheat at 18°C inoculated with this isolate may reflect the large difference in coat protein nucleic acid sequence observed for this isolate compared with others (16).

Only in fall 2001 were symptoms observed in the CO960293 plots following inoculation (Table 5). The significant reduction in yield of CO960293 in 2002 probably relates to the high temperatures in the week following inoculation that year. The average daily maximum temperature for the week following inoculation was 18.5°C for 2000, 31°C for 2002, and 18.8°C for 2003. This heat-induced breakdown in resistance in CO960293, but not in KS96HW10-3 during 2002, also supports our *Wsm1* gene analysis, where we could not demonstrate the presence of the J15 SCAR marker in CO960293. Although KS96HW10-3 resistance to WSMV is temperature sensitive (13), this wheat line did not suffer any yield losses in any year due to WSMV infection. This supports the hypothesis that the resistance in CO960293 is different than that of KS96HW10-3, and that KS96HW10-3 must require slightly higher temperatures of longer duration to induce loss of resistance. The exact temperature conditions (duration of high versus low temperatures) under field conditions at which the resistance is lost has not been determined. Although we demonstrated that the temperature-sensitive resistance was effective in 2 of 3 years for preventing losses to WSMV infection, the use of such resistance probably would be ineffective for growers who use wheat with the dual purpose of grazing and grain production because such wheat usually is planted early, when temperatures are sufficiently high to prevent effective use of the temperature-sensitive resistance should viruliferous WCMs infest the early planted wheat.

In summary, we demonstrated that the temperature-sensitive resistance present in CO960293 was effective in field tests in 2 of 3 years. The temperature-sensitive resistance of this line was demonstrated to be effective at 18°C when inoculated by 51 WSMV isolates collected over time and representing a large geographic area. We also proved that the parental lines of CO960293 are susceptible to WSMV infection at both 18 and 24°C. This indicates that the source of the temperature-sensitive resistance in CO960293 is unknown, but its nature should be elucidated. However, regardless of the source, our data demonstrate that this wheat line can be a valuable source of resistance to WSMV, particularly in areas where temperatures are cool following planting in the fall.

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Registration of CO960293-2 Wheat Germplasm Resistant to *Wheat streak mosaic virus* and Russian Wheat Aphid

CO960293-2 (Reg. no. GP-728, PI 615160) winter wheat (*Triticum aestivum* L.) germplasm was developed by the Colorado Agricultural Experiment Station and jointly released by the Colorado and Kansas Agricultural Experiment Stations in January 2001. CO960293-2 was released because of its resistance to both *Wheat streak mosaic virus* (WSMV) and Russian wheat aphid [RWA; *Diuraphis noxia* (Mordvilko)]. CO960293-2 was developed from the cross PI 222668/'TAM 107'// CO850034 completed in 1991. PI 222668 is a RWA-resistant, winter wheat landrace introduction from east Azerbaijan, Iran (Souza et al., 1991). The pedigree of CO850034, an unreleased experimental line from the Colorado State University breeding program, is Novi Sad 14/Novi Sad 603//'Newton'/3/'Probrand 835'. Segregating populations of the cross from which CO960293 was derived were advanced in the field to the F_4 generation without deliberate selection by bulking successive generations. CO960293 was derived as an $F_{4:5}$ line in 1996 and tested in preliminary yield trials in 1997 and advanced yield trials in 1998. CO960293 was entered in the 1999 Regional Germplasm Observation Nursery (RGON) where it was observed to carry a high level of resistance to WSMV in field tests at Hays, KS. Twenty-five randomly chosen heads were selected from CO960293 in 1998 and grown in the field as headrows in 1999. Selection CO960293-2 (an $F_{7:9}$ line) was homogenous and homozygous for RWA resistance in standard greenhouse screening tests and WSMV resistance in field tests in 2000.

Field tests were conducted at Hays, KS, in 2000 to characterize the WSMV resistance in CO960293. Grain yield data from replicated (n = 4 observations) paired plots (e.g., WSMV-inoculated and control) indicated that WSMV resistance in CO960293 (4050 kg ha^{-1} control vs. 4178 kg ha^{-1} inoculated) is similar to that of the resistant check KS96HW10-3 (4057 kg ha^{-1} control vs. 4077 kg ha^{-1} inoculated) and greater than WSMV resistance in the susceptible checks 'Trego' (4252 kg ha^{-1} control vs. 3290 kg ha^{-1} inoculated) and 'Karl 92' (3492 kg ha^{-1} control vs. 2611 kg ha^{-1} inoculated). Greenhouse RWA resistance evaluations, based on a 1 = very resistant to 5 = very susceptible rating scale, showed that RWA resistance in CO960293-2 (rating = 2) is similar to the resistant check 'Halt' (rating = 2) and greater than the susceptible checks 'Carson' (rating = 5) and 'TAM 107' (rating = 4). Inheritance of RWA resistance in CO960293-2 is not yet known, although RWA resistance in its donor parent PI 222668 was previously determined to be conditioned by either a single dominant or a single dominant and a single recessive gene (Dong et al., 1997).

CO960293-2 is an awned, white-glumed, medium-late maturity, semidwarf winter wheat. Heading date of CO960293-2 is approximately 5 d later than TAM 107 while its plant height is equivalent to TAM 107. While replicated field performance data for CO960293-2 are not available, CO960293 was evaluated in the 1999 Colorado Lower Moisture Variety Trial (LMVT). Data from five replicated dryland locations (n = 15 observations) indicated that CO960293 is yield competitive with commercially available cultivars (TAM 107, 3843 kg ha^{-1}; 'Akron', 3957 kg ha^{-1}; CO960293, 3984 kg ha^{-1}) but has lower grain volume weight (TAM 107, 730 kg m^{-3}; Akron, 735 kg m^{-3}; CO960293, 686 kg m^{-3}).

Greenhouse and growth chamber experiments with the parents of CO960293-2 have failed to identify the original source of the WSMV resistance. Observations of symptom development following mechanical WSMV inoculation in growth chamber experiments indicate that the WSMV resistance in CO960293-2 is temperature-sensitive, similar to the *Wsm1* gene found in CItr 17884, a wheat/*Agropyron intermedium* (Host) P. Beauv. translocation line (Seifers et al., 1995). Chromosome C-banding experiments failed to identify alien chromatin in CO960293 and preliminary data from allelism tests with CO960293 and CItr 17884 indicate independent segregation of the two sources of WSMV resistance.

Small quantities of seed (3 g) for research purposes may be obtained from the corresponding author for at least 5 yr from the date of this publication. Appropriate recognition of the source should be given if this germplasm contributes to the development of improved cultivars or germplasm.

S.D. Haley,* T.J. Martin, J.S. Quick, D.L. Seifers, J.A. Stromberger, S.R. Clayshulte, B.L. Clifford, F.B. Peairs, J.B. Rudolph, J.J. Johnson, B.S. Gill, and B. Friebe

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S.D. Haley, J.S. Quick, J.A. Stromberger, S.R. Clayshulte, B.L. Clifford, and J.J. Johnson, Soil and Crop Sciences Dep., Colorado State Univ., Fort Collins, CO 80523; T.J. Martin and D.L. Seifers, Kansas State Univ. Ag. Res. Center-Hays, Hays, KS 67601; F.B. Peairs and J.B. Rudolph, Bioagricultural Sciences and Pest Management Dep., Colorado State Univ., Fort Collins, CO 80523; B.S. Gill and B. Friebe, Dep. of Plant Pathology, Kansas State Univ., Manhattan, KS 66506.
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*Corresponding author (shaley@lamar.colostate.edu).

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U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL MARKETING SERVICE
SCIENCE AND TECHNOLOGY
PLANT VARIETY PROTECTION OFFICE
BELTSVILLE, MD 20705

Exhibit C

OBJECTIVE DESCRIPTION OF VARIETY
Wheat (*Triticum* spp.)

NAME OF APPLICANT (S)	TEMPORARY OR EXPERIMENTAL DESIGNATION	VARIETY NAME
Kansas Agricultural Exp Staion	KS03HW158	RonL

ADDRESS *(Street and No. or RD No., City, State, Zip Code and Country)*
Kansas State University
Waters Hall
Manhattan KS 66506

FOR OFFICIAL USE ONLY

PVPO NUMBER
#200700244

PLEASE READ ALL INSTRUCTIONS CAREFULLY:

Place the appropriate number that describes the varietal character of this variety in the boxes below. Place a zero in the first box (e.g., [0][9][9] or [0][9]) when number is either 99 or less or 9 or less respectively. Data for quantitative plant characters should be based on a minimum of 100 plants. Comparative data should be determined from varieties entered in the same trial. Royal Horticultural Society or any recognized color standard may be used to determine plant colors; designate system used: RHS. Please answer all questions for your variety; lack of response may delay progress of your application.

1. KIND:

[1]
1 = Common
2 = Durum
3 = Club
4 = Other (Specify) _____

2. VERNALIZATION:

[2]
1 = Spring
2 = Winter
3 = Other (Specify) _____

3. COLEOPTILE ANTHOCYANIN:

[1] 1 = Absent 2 = Present

4. JUVENILE PLANT GROWTH:

[1] 1 = Prostrate 2 = Semi-Erect 3 = Erect

5. PLANT COLOR: (boot stage)

[2]
1 = Yellow-Green
2 = Green
3 = Blue-Green

6. FLAG LEAF: (boot stage)

[2] 1 = Erect 2 = Recurved
[1] 1 = Not Twisted 2 = Twisted
[2] 1 = Wax Absent 2 = Wax Present

7. EAR EMERGENCE:

[1][2][7] Number of Days (Average)
[ ][ ] Number of Days Earlier Than * _____
Same As * Trego
[ ][ ] Number of Days Later Than * _____
**Relative to a PVPO-Approved Commercial Variety Grown in the Same Trial*

8. ANTHER COLOR:

[1] 1 = Yellow 2 = Purple

#200700244

9. PLANT HEIGHT: (from soil to top of head, excluding awns)

[0 6 5] cm (Average)

[ ] cm Taller Than _____ *

Same As _____ *

[0 2] cm Shorter Than Trego _____ *

10. STEM:

A. ANTHOCYANIN

[1] 1 = Absent 2 = Present

B. WAXY BLOOM

[2] 1 = Absent 2 = Present

C. HAIRINESS (last internode of rachis)

[2] 1 = Absent 2 = Present

D. INTERNODE

[1] 1 = Hollow 2 = Semi-Solid 3 = Solid

[4] Number of Nodes

E. PEDUNCLE

[1] 1 = Erect 2 = Recurved 3 = Semi-Erect

[2 5] cm Length

F. AURICLE

[1] Anthocyanin: 1 = Absent 2 = Present

[2] Hair: 1 = Absent 2 = Present

11. HEAD: (At Maturity)

A. DENSITY

[2] 1 = Lax
2 = Middense (Laxidense)
3 = Dense

B. SHAPE

[1] 1 = Tapering
2 = Strap
3 = Clavate
4 = Other (Specify) _____

C. CURVATURE

[2] 1 = Erect
2 = Inclined
3 = Recurved

D. AWNEDNESS

[4] 1 = Awnless
2 = Apically Awnletted
3 = Awnletted
4 = Awned

12. GLUMES: (At Maturity)

A. COLOR

[1] 1 = White
2 = Tan
3 = Other (Specify) _____

B. SHOULDER

[5] 1 = Wanting 2 = Oblique
3 = Rounded 4 = Square
5 = Elevated 6 = Apiculate
7 = Other (Specify) _____

C. SHOULDER WIDTH

[2] 1 = Narrow
2 = Medium
3 = Wide

D. BEAK

[3] 1 = Obtuse
2 = Acute
3 = Acuminate

E. BEAK WIDTH

[2] 1 = Narrow
2 = Medium
3 = Wide

F. GLUME LENGTH

[2] 1 = Short (ca. 7 mm)
2 = Medium (ca. 8 mm)
3 = Long (ca. 9 mm)

G. WIDTH

[2] 1 = Narrow (ca. 3 mm)
2 = Medium (ca. 3.5 mm)
3 = Wide (ca. 4 mm)

H. PUBESCENCE

[2] 1 = Not Present
2 = Present

13. SEED:

A. SHAPE

[1] 1 = Ovate
2 = Oval
3 = Elliptical

B. CHEEK

[2] 1 = Rounded
2 = Angular

C. BRUSH

[2] 1 = Short
2 = Medium
3 = Long

[1] 1 = Not Collared
2 = Collared

D. CREASE

[2] 1 = Width 60% or less of Kernel
2 = Width 80% or less of Kernel
3 = Width Nearly as Wide as Kernel

[3] 1 = Depth 20% or less of Kernel
2 = Depth 35% or less of Kernel
3 = Depth 50% or less of Kernel

E. COLOR #200700244

[1] 1 = White
2 = Amber
3 = Red
4 = Other (Specify) _____

F. TEXTURE

[1] 1 = Hard
2 = Soft
3 = Other (Specify) _____

G. PHENOL REACTION (See Instructions)

[4] 1 = Ivory
2 = Fawn
3 = Light Brown
4 = Dark Brown
5 = Black

H. SEED WEIGHT

[3][2] g/1000 Seed (whole number only)

I. GERM SIZE

[2] 1 = Small
2 = Midsize
3 = Large

14. DISEASE: PLEASE INDICATE THE SPECIFIC RACE OR STRAIN TESTED

(0 = Not Tested 1 = Susceptible 2 = Resistant 3 = Intermediate 4 = Tolerant)

[2]	Stem Rust (*Puccinia graminis* f. sp. *tritici*) TPMK QFCS RSRS RKQQ	[2]	Leaf Rust (*Puccinia recondita* f. sp. *tritici*) MCDS TCTD MFBJ THBJ MJBJ TNRJ
[2]	Stripe Rust (*Puccinia striiformis*)	[1]	Loose Smut (*Ustilago tritici*)
[1]	Tan Spot (*Pyrenophora tritici-repentis*)	[1]	Flag Smut (*Urocystis agropyri*)
[0]	Halo Spot (*Selenophoma donacis*)	[1]	Common Bunt (*Tilletia tritici* or T. *laevis*)
[0]	*Septoria nodorum* (Glume Blotch)	[0]	Dwarf Bunt (*Tilletia controversa*)
[0]	*Septoria avenae* (Speckled Leaf Disease)	[0]	Karnal Bunt (*Tilletia indica*)
[0]	*Septoria tritici* (Speckled Leaf Blotch)	[2]	Powdery Mildew (*Erysiphe graminis* f. sp. *tritici*)
[1]	Scab (*Fusarium* spp.)	[0]	"Snow Molds"
[1]	"Black Point" (Kernel Smudge)	[0]	Common Root Rot (*Fusarium*, *Cochliobolus* and *Bipolaris* spp.)
[1]	Barley Yellow Dwarf Virus (BYDV)	[0]	Rhizoctonia Root Rot (*Rhizoctonia solani*)
[2]	Soilborne Mosaic Virus (SBMV)	[0]	Black Chaff (*Xanthomonas campestris* pv. *translucens*).
[2]	Wheat Yellow (Spindle Streak) Mosaic Virus	[0]	Bacterial Leaf Blight (*Pseudomonas syringae* pv. *syringae*)
[2]	Wheat Streak Mosaic Virus (WSMV)	[ ]	Other (Specify) _____
[ ]	Other (Specify) _____	[ ]	Other (Specify) _____
[ ]	Other (Specify) _____	[ ]	Other (Specify) _____
[ ]	Other (Specify) _____	[ ]	Other (Specify) _____

15. INSECT: (0 = Not Tested 1 = Susceptible 2 = Resistant 3 = Intermediate 4 = Tolerant)

PLEASE SPECIFY BIOTYPE (where needed)

[1]	Hessian Fly (*Mayetiola destructor*)	[ ]	Other (Specify) _____
[0]	Stem Sawfly (*Cephus* spp.)	[ ]	Other (Specify) _____
[0]	Cereal Leaf Beetle (*Oulema melanopa*)	[ ]	Other (Specify) _____

15

15. INSECT: (continued) (0 = Not Tested 1 = Susceptible 2 = Resistant 3 = Intermediate 4 = Tolerant)

#200700244

PLEASE SPECIFY BIOTYPE (Where Needed)

[1]	Russian Aphid (*Diuraphis noxia*)	[ ]	Other (Specify) _____
[1]	Greenbug (*Schizaphis graminum*)	[ ]	Other (Specify) _____
[0]	Aphids	[ ]	Other (Specify) _____

16. ADDITIONAL INFORMATION ON ANY ITEM ABOVE, OR GENERAL COMMENTS:

16

200700244

RonL, PVP Application
Exhibit D: Additional Description of the Variety

RonL (KS03HW158) is a hard white winter wheat developed by the Kansas Agricultural Experiment Station. It was selected from the cross Trego/CO960293 which was made at Hays, KS in 1999. The pedigree method of breeding was used during its development. RonL is an increase of a F_6 head-row selected in 2003.

It was tested in the Kansas Intra-State Nursery (KIN) over the last two years and preformed best at the western Kansas locations. Its primary area of adaptation is very similar to that of Trego. The milling and baking tests on RonL indicate that it has good baking quality. In 2005 the Wheat Quality Council judged it as being well above average in overall baking quality and not significantly different from the bread quality of Jagger.

RonL is an awned, white chaffed semi-dwarf with a medium late maturity similar to Trego. Its height is also similar to that of Trego. Seed of RonL are white with an ovate shape and medium sized brush. Variants within RonL include tall plants, red chaffed plants, and plants with red seed but none of these occur at a frequency of more than one in 10,000 plants.

RonL carries the temperature sensitive high level of resistance to wheat streak mosaic derived from CO960293. It is also resistant to soilborne mosaic virus and stripe rust. It is susceptible to the current races of leaf rust and Hessian fly.

Seed stocks will be maintained by intensely rogueing foundation production fields and by re-purification through head rows at the KSU Agricultural Research Center-Hays. Foundation, Registered, and Certified classes of seed will be recognized. Plant Variety Protection has been applied for and the "Certification Option" elected.

The following is the milling and baking summary for RonL comparing it to the check variety Jagger done by members of the Wheat Quality Council in 2004 and 2005. The Wheat Quality Council is made up of members representing most of the major milling and baking companies in the US.

200700244

Description of Test Plots and Breeder Entries

KANSAS-Hays – Joe Martin

The samples submitted were grown at a bottomland site at Hays in 2004. The nursery was not fertilized . Yield levels were good in spite of the drought stress in early spring. Diseases or insects were not a problem however the samples were rained on prior to harvest and some sprout damage was noticed.

Jagger (check)

2137 (check)

KS02HW34 and KS02HW35

These lines are hard white sister lines selected from the cross Trego/Jagger 8W. Jagger 8W was a hard white selection made from Jagger at the same time Betty was selected. These lines have been our top performers in western Kansas dryland nurseries over the last three years. They both have significant improvements over Trego. Both are resistant to stripe rust and leaf rust. They also have an improved level of pre-harvest sprouting tolerance. In our sprouting tests the last three years they have been equal to the red wheat Jagger. KS02HW34 was tested in the 2003 by the Wheat Quality Council and has been approved for possible release to seed producers in 2005.

KS03HW158

This line is a hard white selection from the cross Trego/CO960293. It is basically a Trego type wheat which carries the resistance to wheat streak mosaic virus from CO960293. KS03HW158 has had improved mixing strength and absorption relative to Trego in the Wheat Quality Labs bake tests at KSU. The earliest this line could be released would be 2006.

200700244

KANSAS-Hays: 2004 (Small-Scale) Samples[a]

Test entry number	04-2401	04-2402	04-2403	04-2404	04-2405
Sample identification	Jagger (check)	2137 (check)	KS02HW34	KS02HW35-5	KS03HW158
Wheat Data					
FGIS classification	HRW ?	HRW	HDWH	HDWH	HDWH
Test weight (lb/bu)	56.8	57.4	60.8	61.1	60.4
Hectoliter weight (kg/hl)	74.8	75.6	80.0	80.4	79.5
1000 kernel weight (gm)	27.8	31.2	29.3	33.0	33.2
NIR hardness	58.0	53.0	57.0	65.0	71.0
Wheat kernel size test (Rotap)					
Over 7 wire (%)	59.5	66.8	59.4	76.1	82.1
Over 9 wire (%)	40.3	33.1	40.6	23.9	17.9
Through 9 wire (%)	0.2	0.1	0.0	0.0	0.1
Single kernel analysis (skcs)					
Hardness/s.d. hardness	66.0/15.0	51.0/14.0	63.0/14.0	70.0/14.0	64.0/17.0
Weight (mg)/s.d. weight	31.0/9.9	32.3/7.9	31.3/7.6	33.2/7.7	34.8/8.7
Diameter (mm)/s.d. diameter	2.39/0.51	2.37/0.42	2.35/0.41	2.46/0.44	2.57/0.53
SKCS distribution	02-04-20-74	10-29-34-27	03-08-27-62	01-02-17-80	04-15-15-66
Classification	Hard	Hard	Hard	Hard	Hard
Wheat moisture (%)	10.0	10.1	10.2	10.2	10.2
Wheat protein (12% mb)	15.0	14.0	14.0	14.2	14.3
Wheat ash (12% mb)	1.45	1.40	1.27	1.28	1.33
Milling and Flour Quality Data					
Flour yield (%, str. grade)	75.0	72.7	74.4	74.2	73.2
Flour moisture (%)	12.9	12.8	12.6	12.6	11.5
Flour protein (14% mb)	14.0	12.2	12.4	12.4	12.6
Flour ash (14% mb)	0.36	0.34	0.31	0.32	0.30
Glutomatic					
Wet gluten (%)	37.0	34.4	36.6	35.4	34.8
Dry gluten (%)	13.4	12.4	12.5	12.4	12.3
Gluten index	99	97	90	97	97
Flour color					
Agtron flour color	72	77	76	74	74
Simon/Kent-Jones flour color	0.76	-1.56	2.46	-0.98	-1.37
Minolta Color Meter					
L*	91.08	92.10	92.0	91.51	91.59
a*	-1.35	-1.37	-1.39	-1.43	-1.68
b*	8.49	6.83	7.21	7.29	8.47
Falling number (sec)	511	500	407	419	283
Flour particle size (avg. μ)					
Fisher sub sieve sizer	19.0	17.5	19.0	20.0	20.0

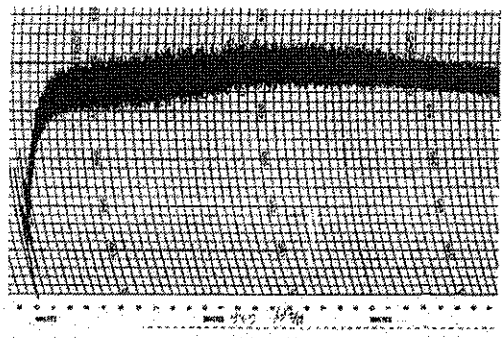
[a] s.d. = standard deviation; skcs = Single Kernel Characterization System 4100.

200700244

Physical Dough Tests

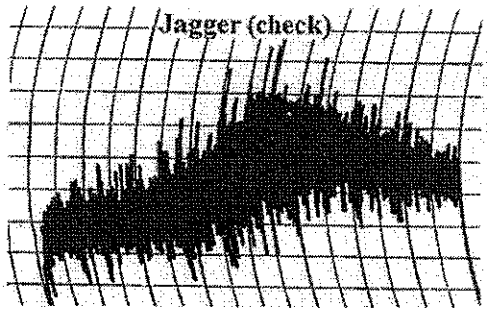
2004 (Small Scale) KANSAS-Hays

Farinograms



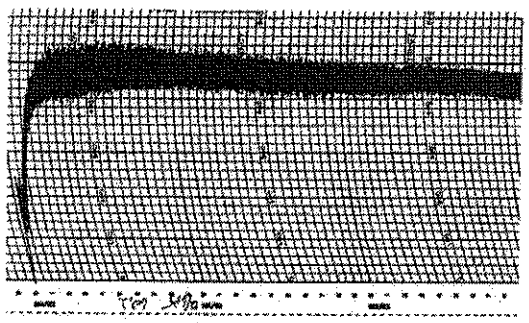
Abs. 61.8%, Peak 16.3 min, Stab. 16.6 min

Mixograms

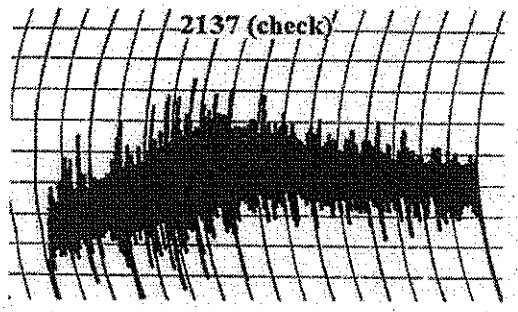


Abs. 64.6%, Mix time 4.6 min

04-2401, Jagger (check)



Abs. 61.8%, Peak 11.0 min, Stab. 27.6 min



Abs. 62.1% Mix Time 3.4 min

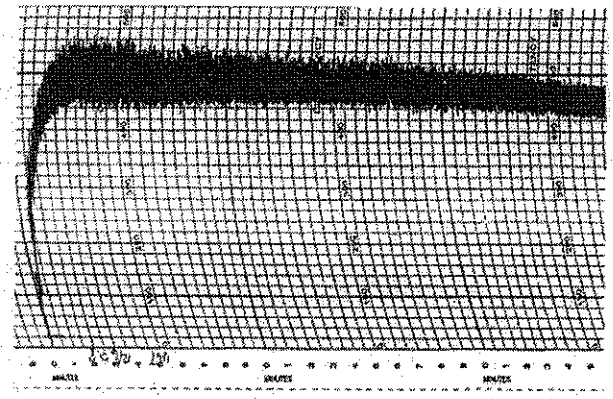
04-2402, 2137 (check)

2007002744

Physical Dough Tests

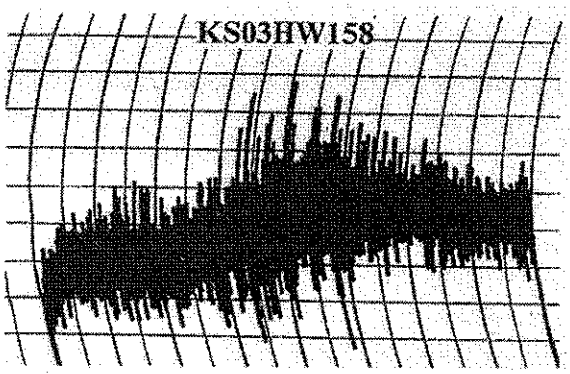
2004 (Small Scale) KANSAS-Hays (continued)

Farinograms



Abs. 58.7%, Peak 13.7 min, Stab. 24.5 min

Mixograms



Abs. 61.4%, Mix time 4.8 min

04-2405. KS03HW158

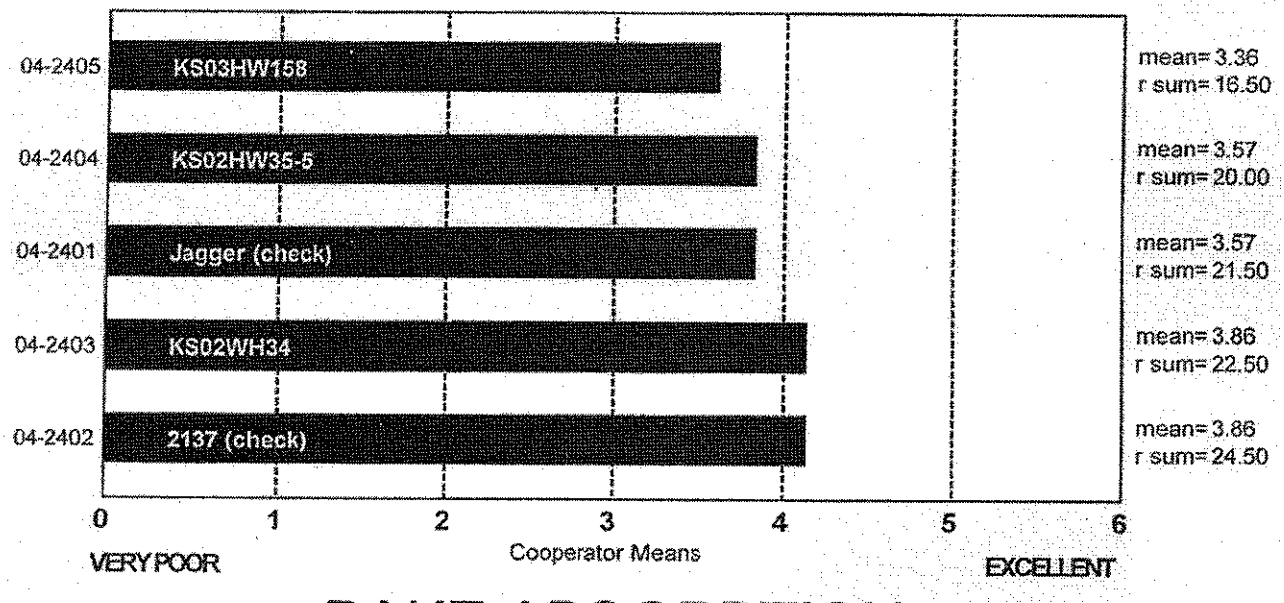
2007002244

SPONGE CHARACTERISTICS

(Small Scale) Kansas-Hays

ncoop=7
chisq=2.06
chisqc=4.97
cvchisq=9.49
crdiff=

Variety order by rank sum.
No samples different at 5.0% level of significance.

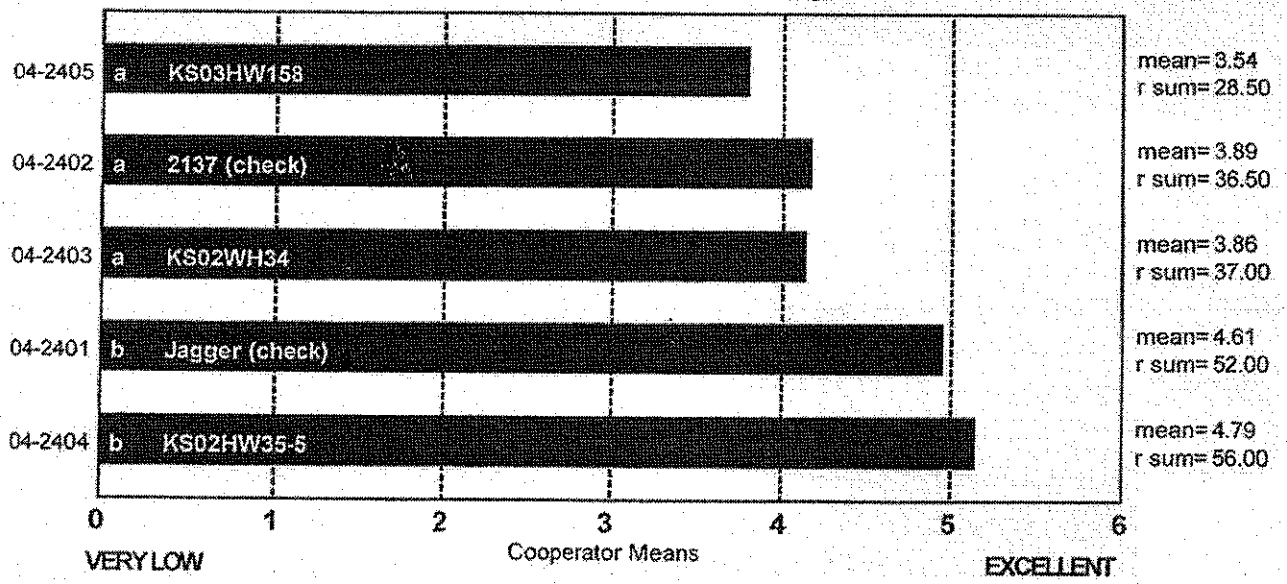


BAKE ABSORPTION

(Small Scale) Kansas-Hays

ncoop=14
chisq=15.24
chisqc=22.58
cvchisq=9.49
crdiff=11.06

Variety order by rank sum.
Samples with the same letter not different at 5.0% level of significance.



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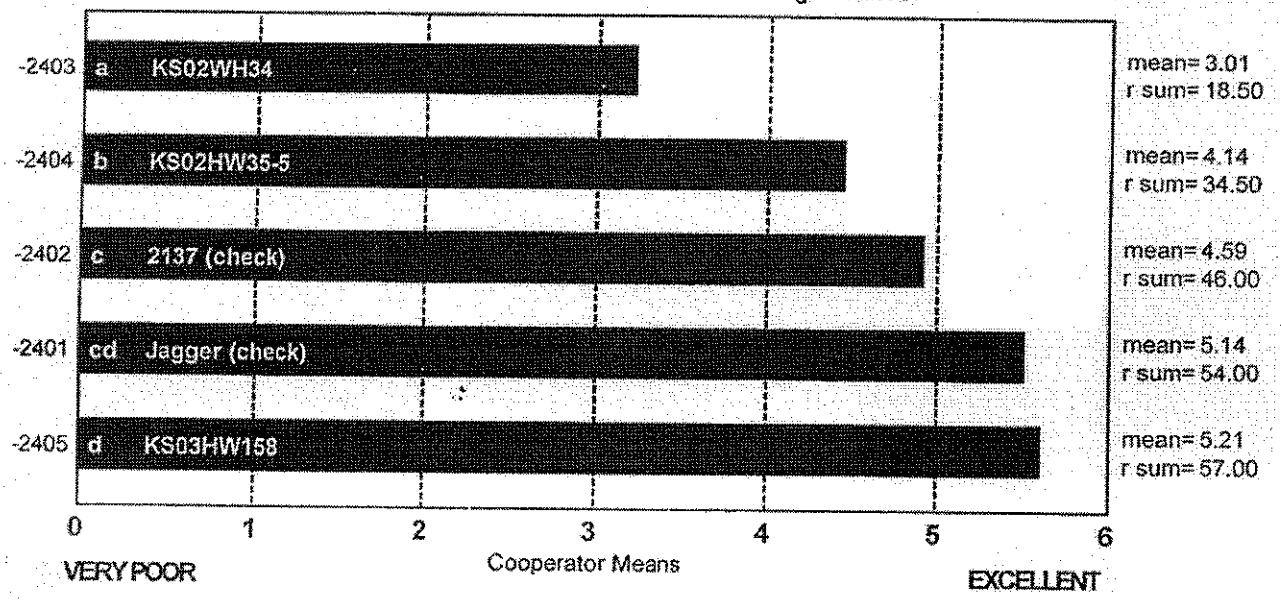
BAKE MIX TIME

(Small Scale) Kansas-Hays

Variety order by rank sum.
Samples with the same letter not different at 5.0% level of significance.

ncoop= 14
chisq= 28.39
chisqc= 32.44
cvchisq= 9.49
crdiff= 10.57

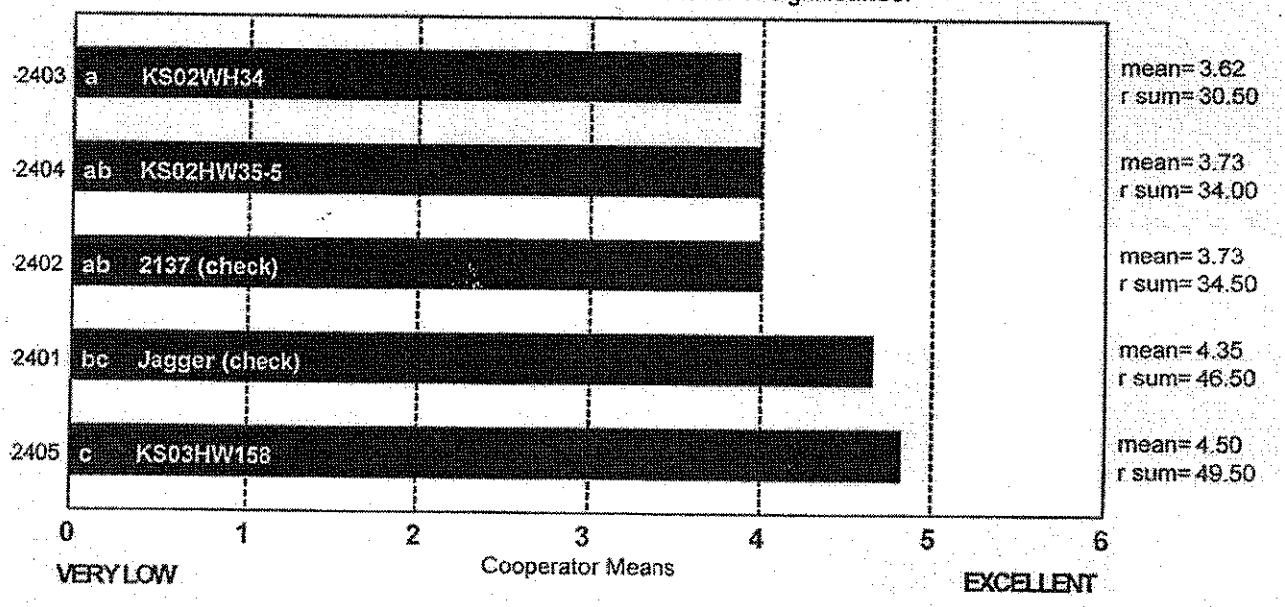


MIX TOLERANCE

(Small Scale) Kansas-Hays

Variety order by rank sum.
Samples with the same letter not different at 5.0% level of significance.

ncoop= 13
chisq= 8.74
chisqc= 11.36
cvchisq= 9.49
crdiff= 13.08



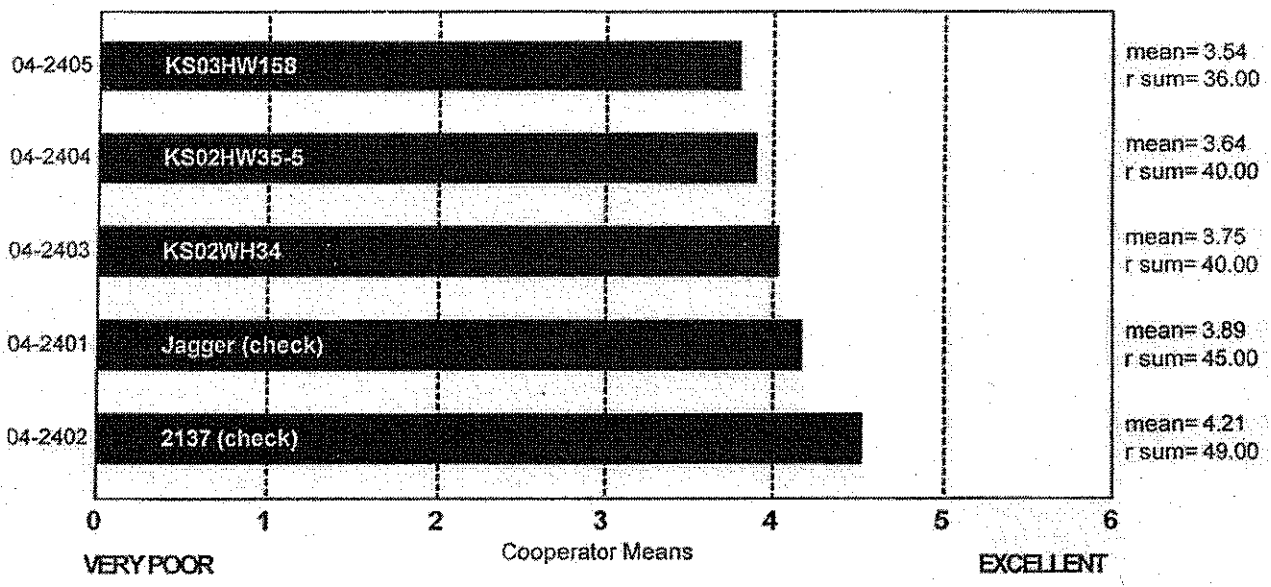
200700244

DOUGH CHAR. 'OUT OF MIXER'

(Small Scale) Kansas-Hays

ncoop= 14
chisq= 2.91
chisqc= 3.74
cvchisq= 9.49
crdiff=

Variety order by rank sum.
No samples different at 5.0% level of significance.



DOUGH CHAR. 'OUT OF MIXER', DESCRIBED

(Small Scale) Kansas-Hays

	Sticky	Wet	Tough	Good	Excellent
04-2401 Jagger (check)	0	0	6	6	2
04-2402 2137 (check)	0	0	4	7	3
04-2403 KS02WH34	2	0	1	11	0
04-2404 KS02HW35-5	0	0	3	11	0
04-2405 KS03HW158	0	0	5	7	2

Frequency Table

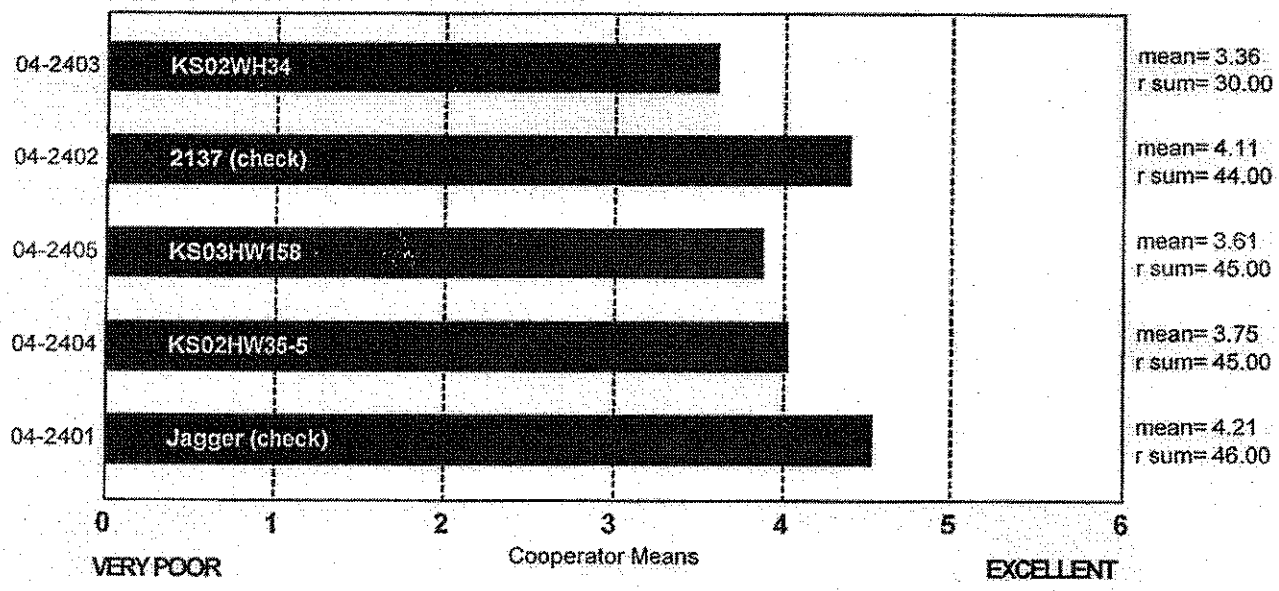
200700244

DOUGH CHAR. 'AT MAKE UP'

(Small Scale) Kansas-Hays

ncoop= 14
chisq= 5.20
chisqc= 6.17
cvchisq= 9.49
crdiff=

Variety order by rank sum.
No samples different at 5.0% level of significance.



DOUGH CHAR. 'AT MAKE UP', DESCRIBED

(Small Scale) Kansas-Hays

	Sticky	Wet	Tough	Good	Excellent
04-2401 Jagger (check)	0	0	6	5	3
04-2402 2137 (check)	0	0	4	7	3
04-2403 KS02WH34	2	2	3	6	1
04-2404 KS02HW35-5	0	0	3	9	2
04-2405 KS03HW158	0	0	5	8	1

Frequency Table

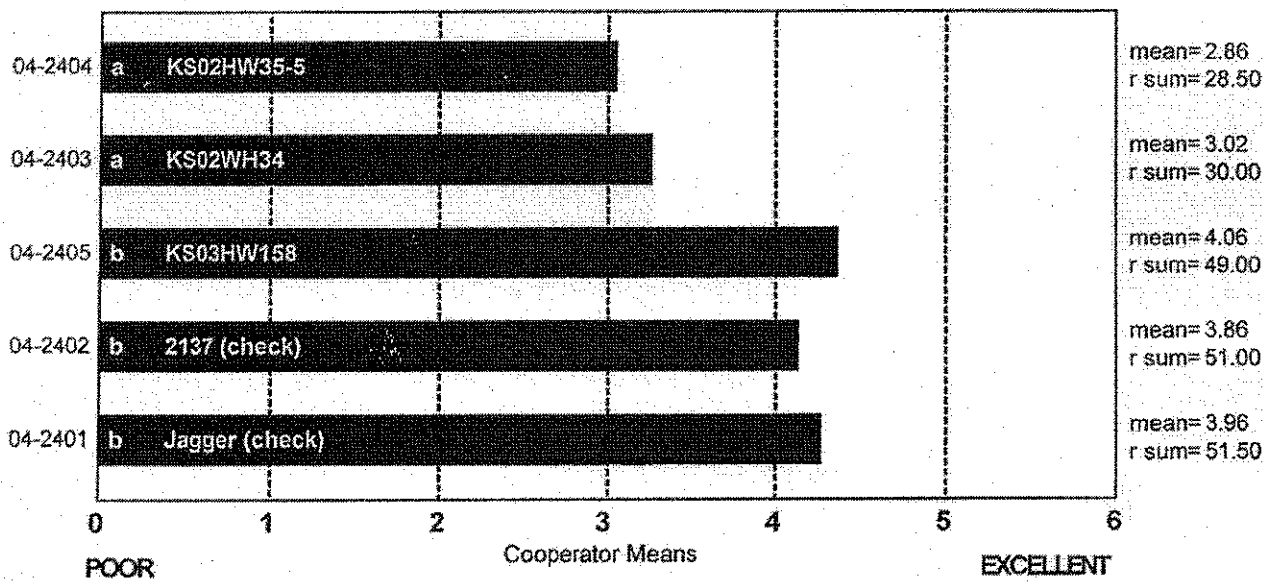
200700244

CRUMB GRAIN

(Small Scale) Kansas-Hays

ncoop= 14
chisq= 15.61
chisqc= 17.28
cvchisq= 9.49
crdiff= 13.77

Variety order by rank sum.
Samples with the same letter not different at 5.0% level of significance.



CRUMB GRAIN, DESCRIBED

(Small Scale) Kansas-Hays

	Open	Fine	Dense
04-2401 Jagger (check)	7	7	0
04-2402 2137 (check)	2	10	2
04-2403 KS02WH34	10	3	0
04-2404 KS02HW35-5	9	4	0
04-2405 KS03HW158	6	6	1

Frequency Table

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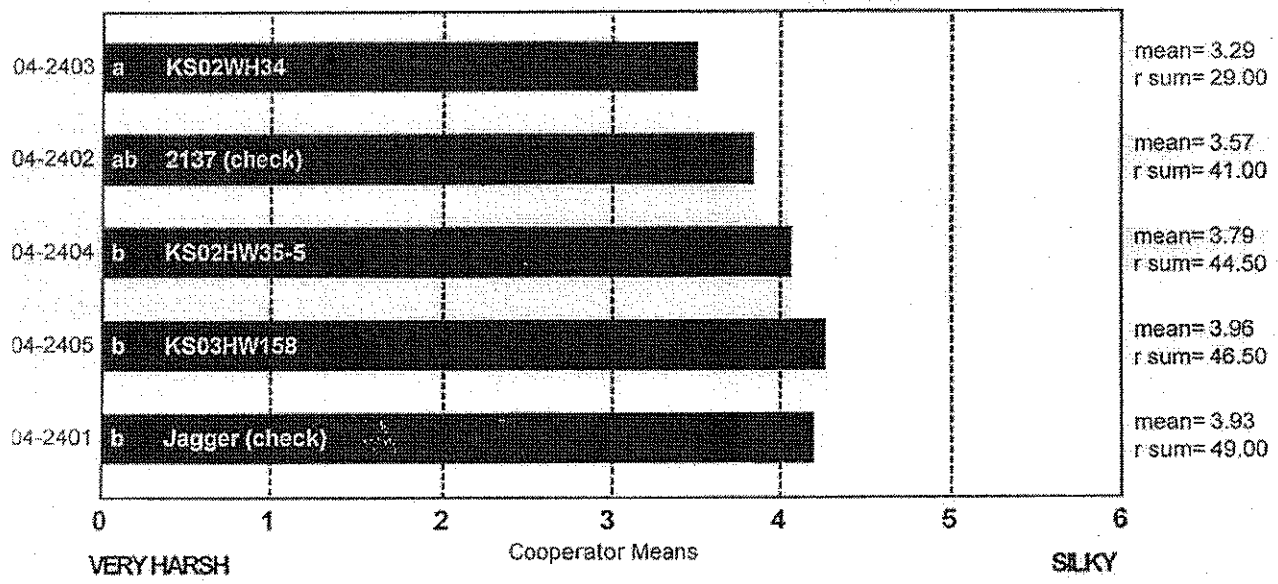
2007002444

CRUMB TEXTURE

(Small Scale) Kansas-Hays

ncoop= 14
chisq= 7.01
chisqc= 9.53
cvchisq= 9.49
crdiff= 13.61

Variety order by rank sum.
Samples with the same letter not different at 5.0% level of significance.



CRUMB TEXTURE, DESCRIBED

(Small Scale) Kansas-Hays

	Harsh	Smooth	Silky
04-2401 Jagger (check)	1	10	3
04-2402 2137 (check)	4	7	3
04-2403 KS02WH34	6	7	1
04-2404 KS02HW35-5	5	5	4
04-2405 KS03HW158	2	9	3

Frequency Table

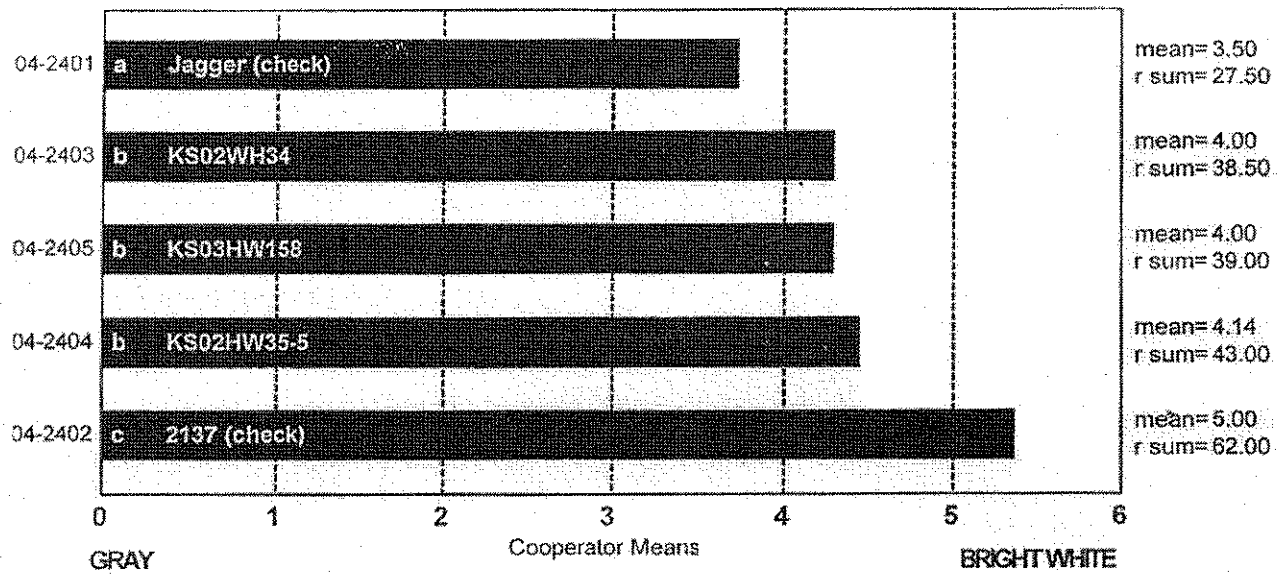
2007002744

CRUMB COLOR

(Small Scale) Kansas-Hays

ncoop= 14
chisq= 18.07
chisqc= 25.69
cvchisq= 9.49
crdiff= 10.75

Variety order by rank sum.
Samples with the same letter not different at 5.0% level of significance.



CRUMB COLOR, DESCRIBED

(Small Scale) Kansas-Hays

	Gray	Yellow	Dull	Creamy	Bright White
04-2401 Jagger (check)	1	1	2	10	0
04-2402 2137 (check)	0	0	0	3	5
04-2403 KS02WH34	0	1	0	10	0
04-2404 KS02HW35-5	0	0	3	5	1
04-2405 KS03HW158	0	0	1	8	1

Frequency Table

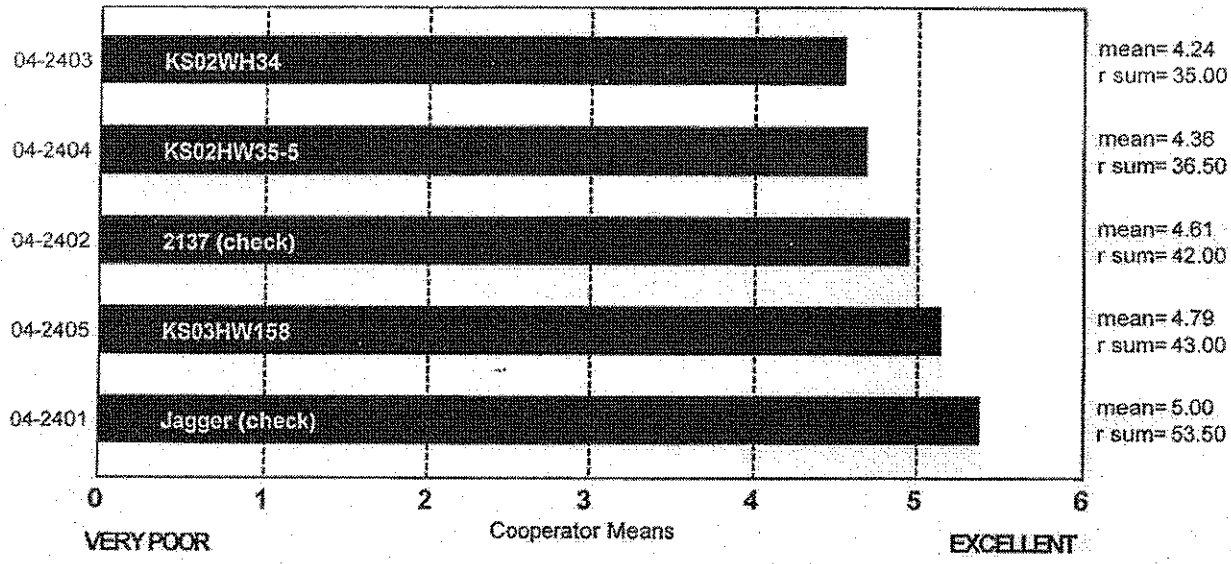
200700244

LOAF VOLUME

(Small Scale) Kansas-Hays

ncoop= 14
chisq= 6.07
chisqc= 7.39
cvchisq= 9.49
crdiff=

Variety order by rank sum.
No samples different at 5.0% level of significance.

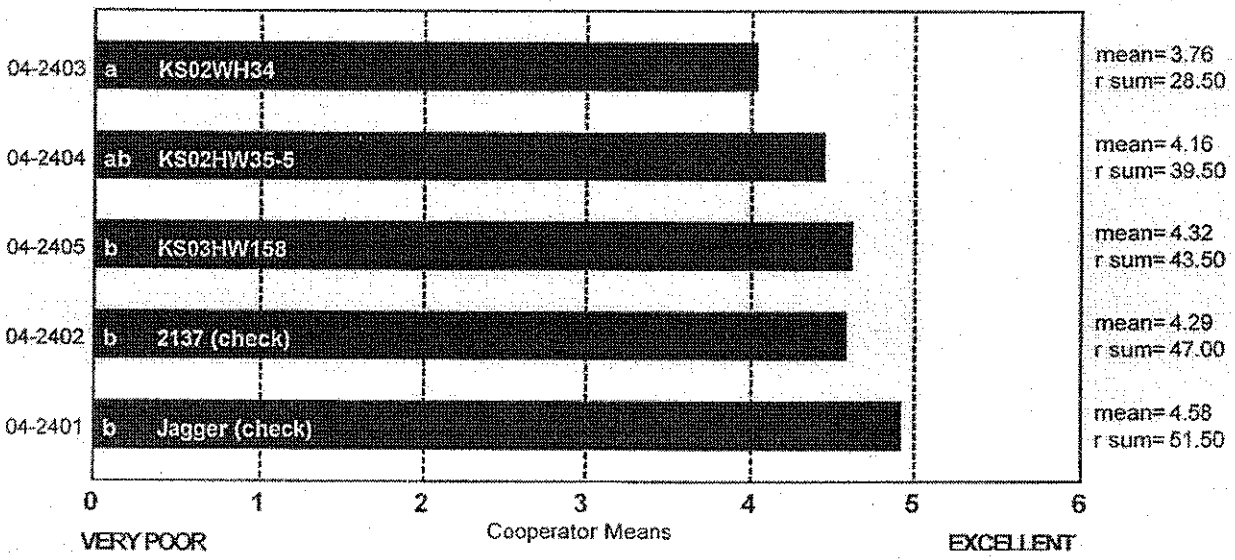


OVERALL BAKING QUALITY

(Small Scale) Kansas-Hays

ncoop= 14
chisq= 8.74
chisqc= 10.83
cvchisq= 9.49
crdiff= 14.06

Variety order by rank sum.
Samples with the same letter not different at 5.0% level of significance.



200700244

Description of Test Plots and Breeder Entries

Kansas-Hays – reported by Joe Martin

The samples submitted were grown at a bottomland site at Hays in 2005. The nursery was not fertilized. The yield of the 2137 check was affected by the stripe rust. The other entries were slightly affected by leaf rust.

Jagger (check)

2137 (check)

KS03HW158

This line is a hard white selection from the cross Trego/CO960293. It is basically a Trego type wheat which carries the resistance to wheat streak mosaic virus from CO960293. KS03HW158 has had improved mixing strength and absorption relative to Trego in the Wheat Quality Labs bake tests at KSU. This line was also tested by the Wheat Quality Council in 2005. It could be released next summer.

KS03HW6-6

This line is a hard white selection from the following cross, FS4 /KS97HW150 //KS97HW349/3/Trego. This line is also a Trego type line that carries the BASF gene for resistance to their herbicide Beyond. This line has baked better than Trego in KSU tests. A limited release could be made in 2006.

2004002244

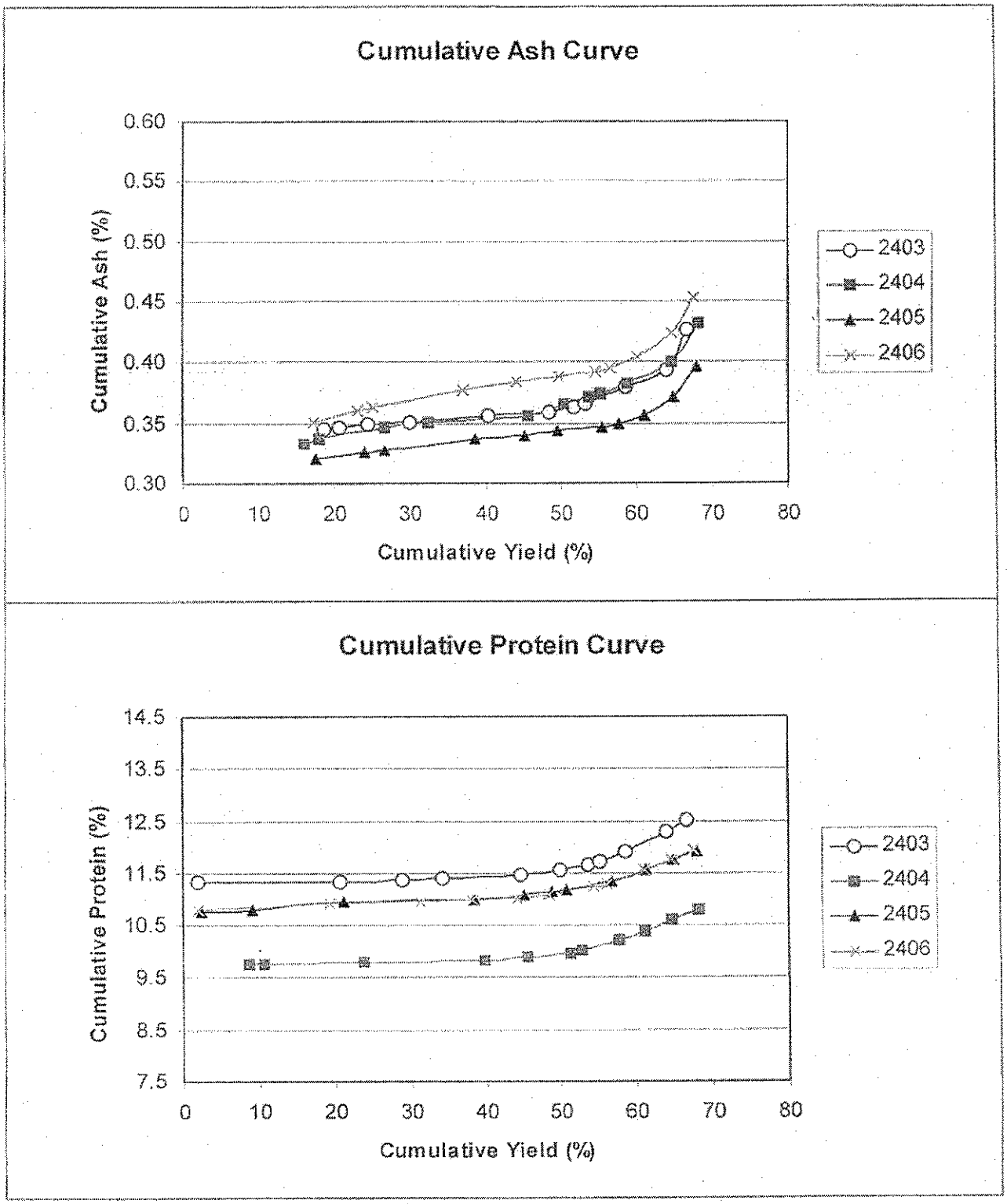
Kansas-Hays: 2005 (Small-Scale) Samples[a]

Test entry number	05-2403	05-2404	05-2405	05-2406
Sample identification	Jagger(check)	2137(check)	KS03HW6-6	KS03HW158-1
Wheat Data				
FGIS classification	HRW	HRW	HDWH	HDWH
Test weight (lb/bu)	60.2	60.6	60.6	59.3
Hectoliter weight (kg/hl)	79.2	79.7	79.7	78.0
1000 kernel weight (gm)	27.2	25.0	27.3	27.0
NIR hardness	77.5	85.0	65.2	79.1
Wheat kernel size (Rotap)				
Over 7 wire (%)	55.4	43.0	43.5	55.0
Over 9 wire (%)	43.6	54.7	55.5	43.8
Through 9 wire (%)	1.0	2.3	1.0	1.2
Single kernel analysis (skcs)				
Hardness/s.d. hardness	73.0/16.8	76.6/14.5	64.7/15.8	77.7/15.1
Weight (mg)/s.d. weight	29.0/8.4	27.3/7.7	28.9/8.5	29.1/8.5
Diameter (mm)/s.d. diameter	2.17/0.47	2.09/0.45	2.08/0.46	2.12/0.46
SKCS distribution	01-06-11-82	01-02-08-89	02-11-22-65	01-01-10-88
Classification	Hard	Hard	Hard	Hard
Wheat moisture (%)	10.3	10.4	10.7	11.4
Wheat protein (12% mb)	13.8	12.3	13.3	13.7
Wheat ash (12% mb)	1.47	1.43	1.48	1.58
Milling and Flour Quality Data				
Flour yield (%, straight grade)	72.4	71.7	71.0	70.7
Flour moisture (%)	11.7	11.7	12.0	11.1
Flour protein (14% mb)	12.3	10.6	11.7	11.9
Flour ash (14% mb)	0.45	0.44	0.41	0.47
Glutomatic				
Wet gluten (%)	35.0	29.4	33.1	33.6
Dry gluten (%)	12.2	10.5	11.7	11.9
Gluten index	96.9	95.6	96.4	97.9
Flour color				
Agtron flour color	76.5	81.0	83.0	79.0
Simon/Kent-Jones flour color	-0.26	-1.26	-0.32	-0.36
Minolta color meter				
L*	90.80	91.12	91.35	90.73
a*	-1.39	-1.54	-1.49	-1.85
b*	8.61	8.46	8.34	10.09
Falling number (sec)	480	438	452	414
Particle size (avg. micron)				
Fisher sub sieve sizer	21.5	11.5	9.8	11.8

[a]s.d. = standard deviation; skcs = Single Kernel Characterization System 4100.

200700244

Kansas - Hays: Cumulative Ash and Protein Curves for 2005 (Small-Scale) Samples



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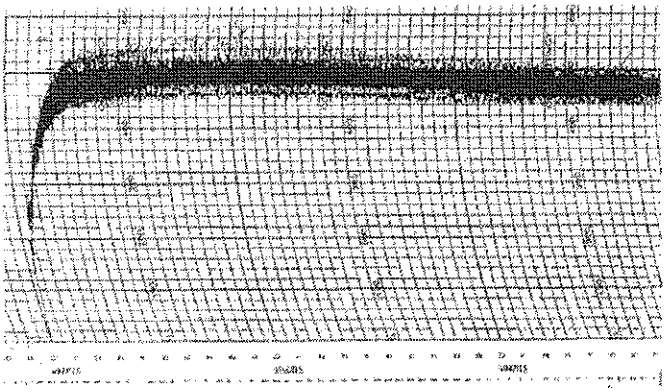
Physical Dough Tests 200700244

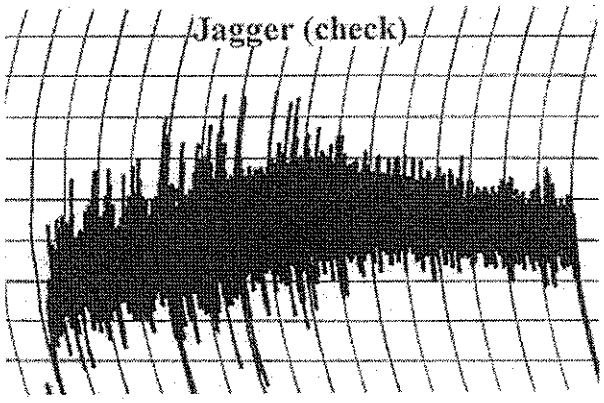
2005 (Small Scale) Samples – Kansas-Hays

Farinograms



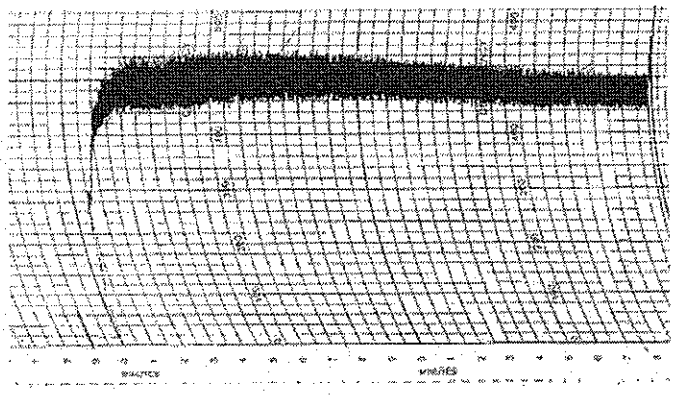
Abs. 62.7%, Peak 10.0 min, Stab. 15.0 min

Mixograms

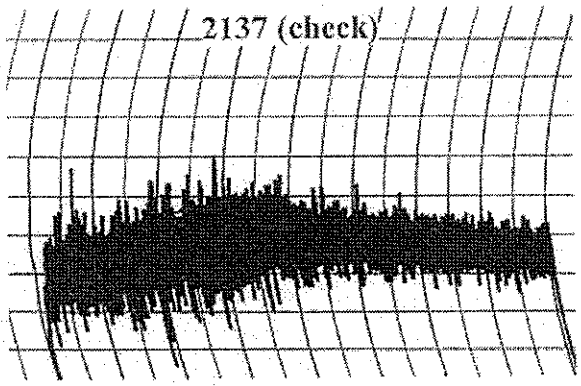


Abs. 63.6%, Mix time 4.1 min

05-2403, Jagger (check)



Abs. 60.1%, Peak 7.5 min, Stab. 15.8 min



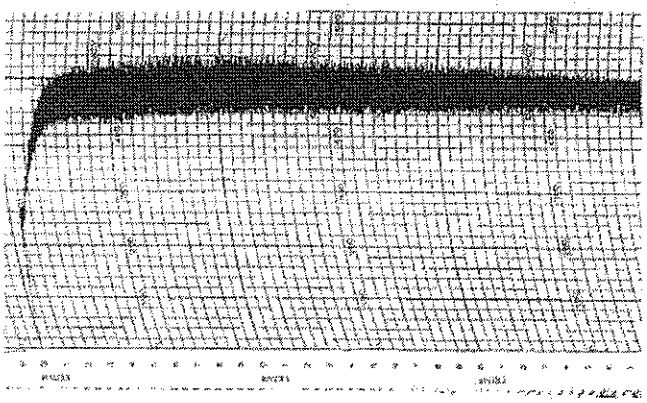
Abs. 61.4%, Mix time 3.5 min

05-2404, 2137 (check)

Physical Dough Tests 2007002444

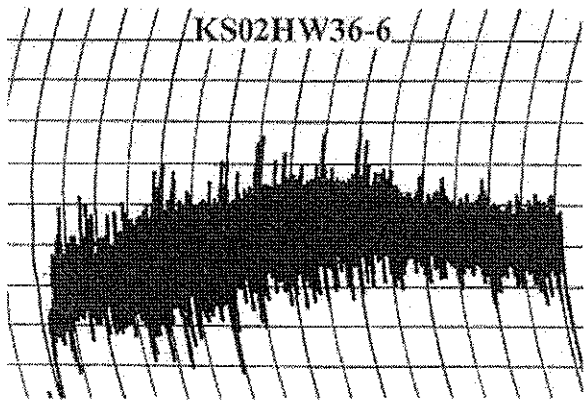
2005 (Small Scale) Samples – Kansas-Hays

Farinograms



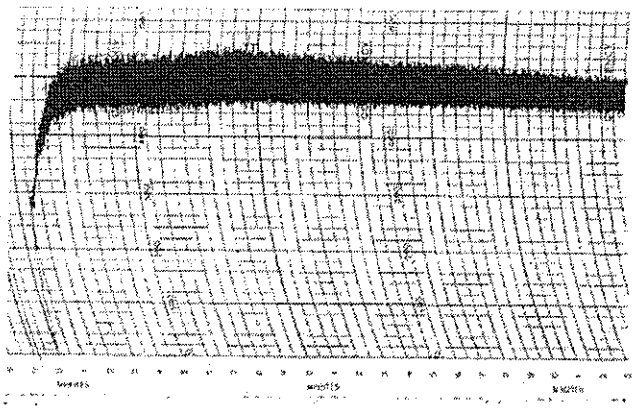
Abs. 60.7%, Peak 13.0 min, Stab. 32.3 min

Mixograms

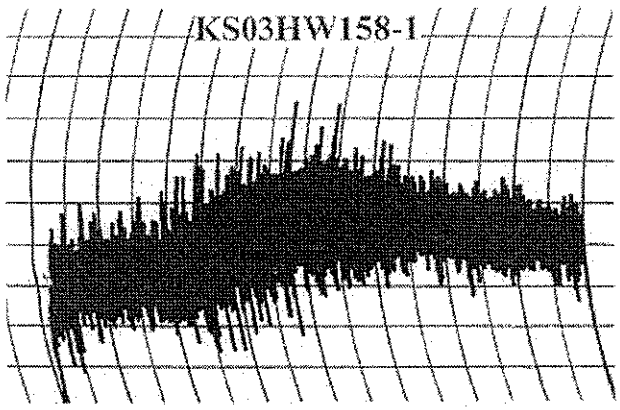


Abs. 63.2%, Mix time 4.8 min

05-2405, KS03HW6-6



Abs. 61.5%, Peak 9.5 min, Stab. 22.5 min

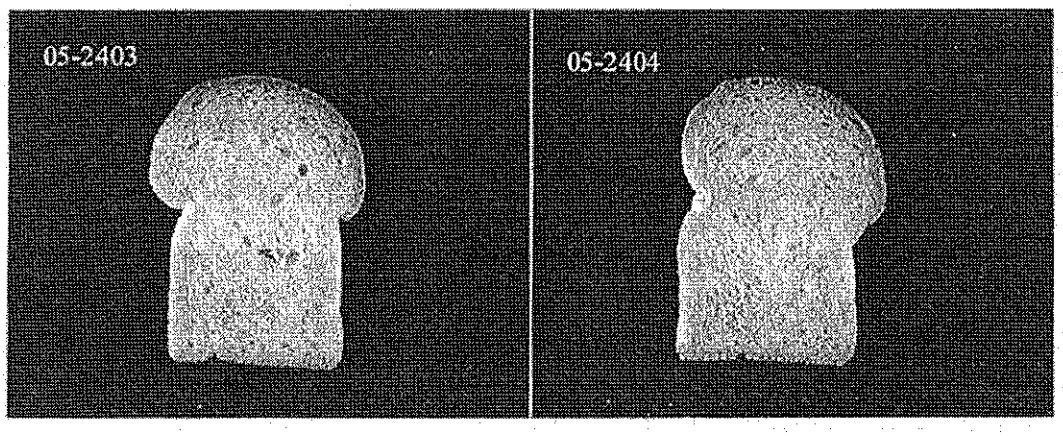


Abs. 63.7%, Mix time 4.4 min

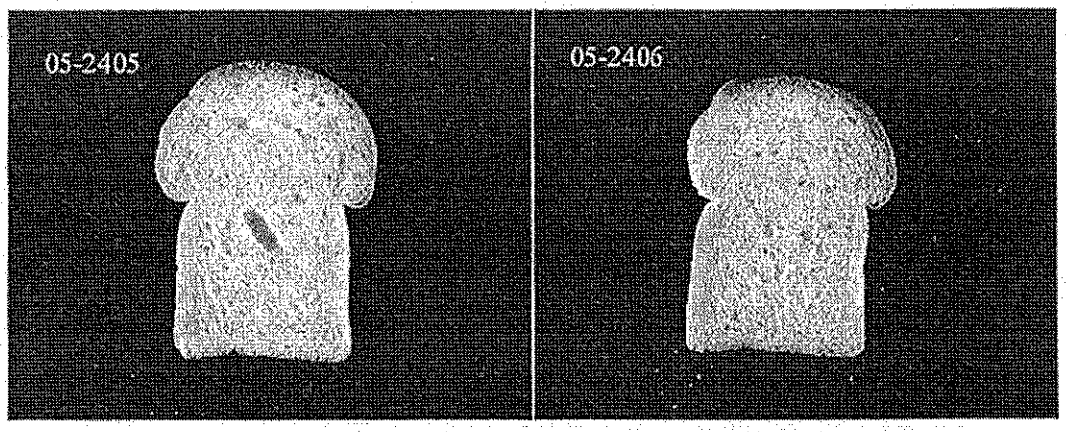
05-2406, KS03HW158-1

2007002244

Kansas - Hays: C-Cell Bread Images and Analysis for 2005 (Small-Scale) Samples



Entry #	Slice Brightness	Number Cells	Number Holes	Wall Thickness	Cell Diameter
2403	153.05	3953.0	0.63	3.08	13.28
2404	157.18	3954.5	1.05	3.08	12.91



Entry #	Slice Brightness	Number Cells	Number Holes	Wall Thickness	Cell Diameter
2405	153.64	3798.5	1.31	3.21	15.03
2406	153.13	3945.5	0.53	3.09	13.28

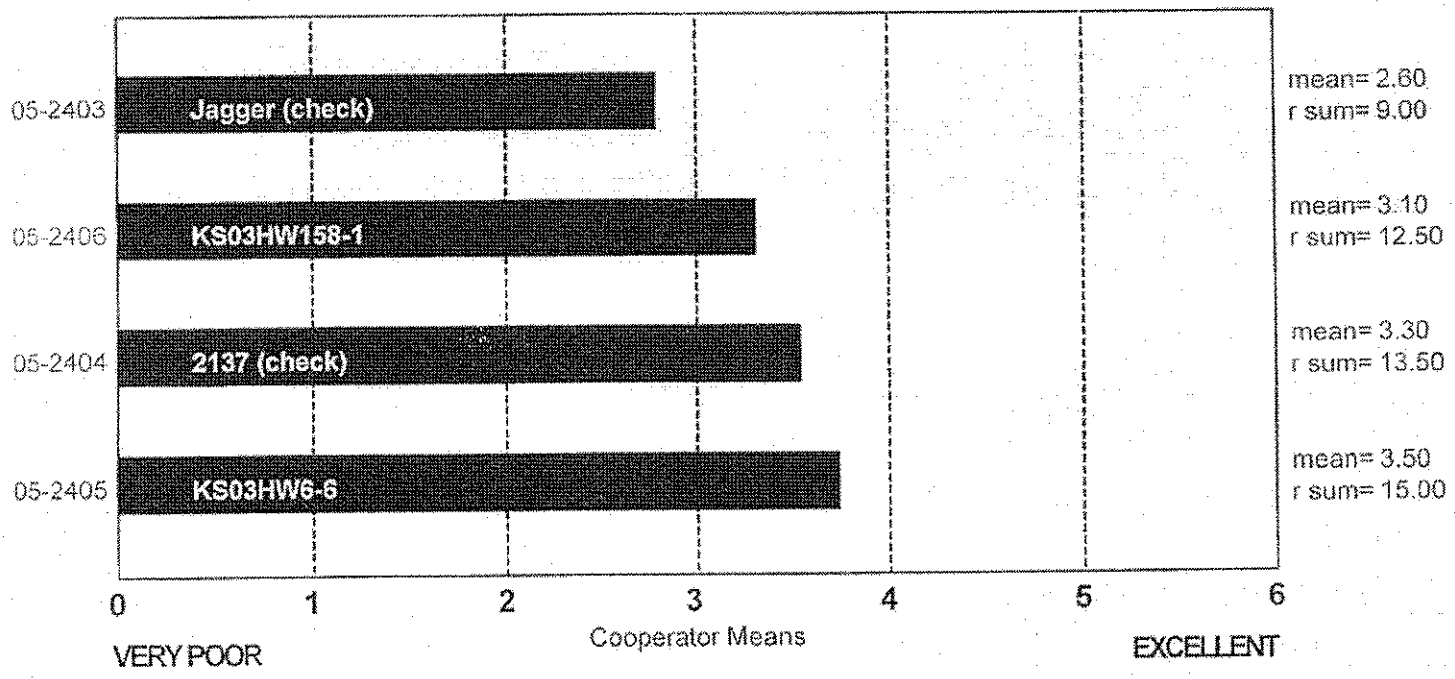
2007002444

SPONGE CHARACTERISTICS

(Small Scale) Kansas-Hays

ncoop= 5
chisq= 2.34
chisqc= 3.44
cvchisq= 7.82
crdiff=

Variety order by rank sum.
No samples different at 5.0% level of significance.

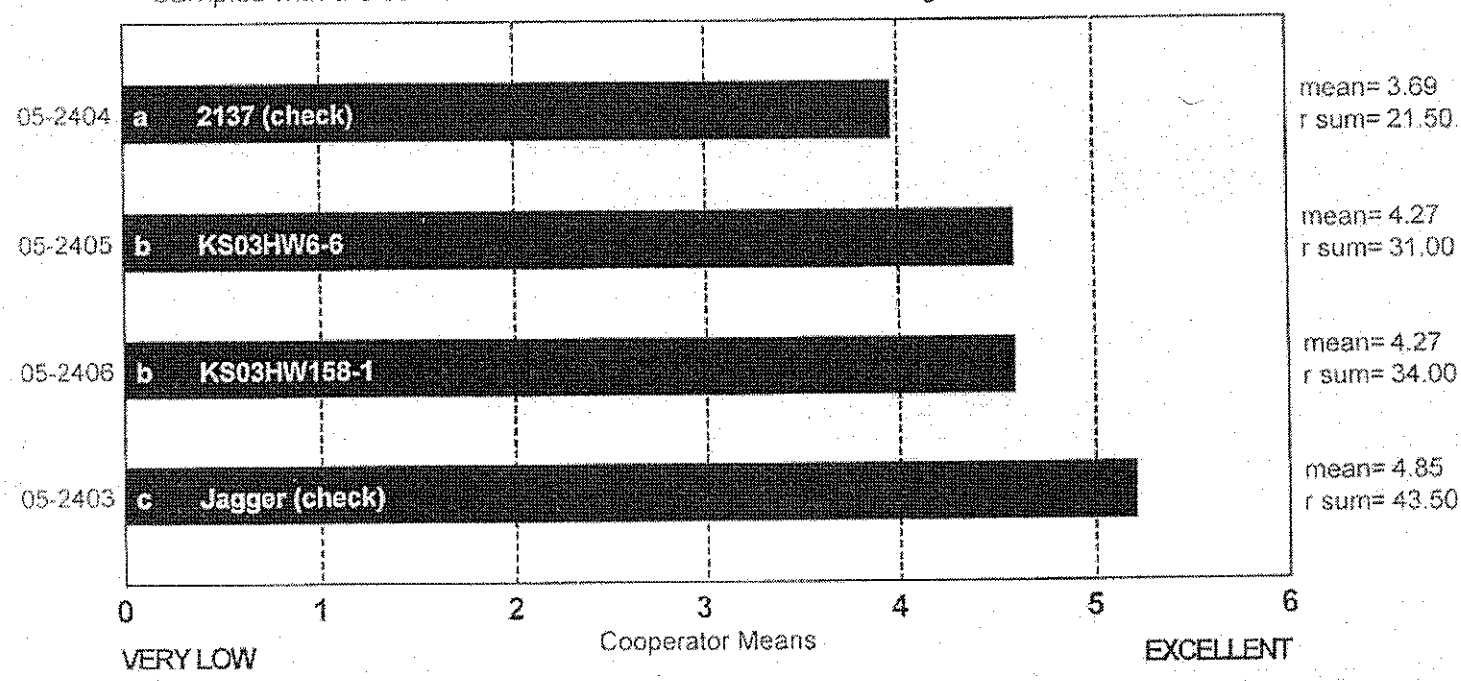


BAKE ABSORPTION

(Small Scale) Kansas-Hays

ncoop= 13
chisq= 11.38
chisqc= 18.26
cvchisq= 7.82
crdiff= 8.00

Variety order by rank sum.
Samples with the same letter not different at 5.0% level of significance.



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BAKE ABSORPTION, ACTUAL (14% MB)

(Small Scale) Kansas-Hays

	Coop. A	Coop. B	Coop. C	Coop. D	Coop. E	Coop. F	Coop. G	Coop. H	Coop. I	Coop. J	Coop. K	Coop. L	Coop. M
05-2403 Jagger (check)	67.0	66.0	59.7	64.7	64.0	77.2	65.6	66.0	61.2	59.0	62.0	66.5	63.6
05-2404 2137 (check)	62.5	64.0	57.1	63.6	63.0	73.2	65.4	61.0	58.6	57.0	60.0	64.5	59.3
05-2405 KS03HW6-6	66.0	64.0	57.7	63.2	63.0	75.2	65.2	63.0	59.2	58.0	61.0	64.5	62.3
05-2406 KS03HW158-1	65.0	64.0	58.5	64.0	65.0	75.2	65.7	61.0	60.0	59.0	62.0	65.0	62.8

Raw Data

BAKE MIX TIME, ACTUAL

(Small Scale) Kansas-Hays

	Coop. A	Coop. B	Coop. C	Coop. D	Coop. E	Coop. F	Coop. G	Coop. H	Coop. I	Coop. J	Coop. K	Coop. L	Coop. M
05-2403 Jagger (check)	3.5	6.0	8.0	3.0	6.0	4.1	5.5	28.0	2.8	20.0	6.0	18.0	5.5
05-2404 2137 (check)	3.3	3.0	7.0	2.8	6.0	4.4	4.0	15.0	2.8	15.0	3.0	10.0	5.9
05-2405 KS03HW6-6	3.7	8.0	8.0	3.3	6.0	4.3	5.0	20.0	3.0	18.0	6.0	18.0	5.6
05-2406 KS03HW158-1	4.3	6.0	8.0	3.0	6.2	4.4	5.5	25.0	3.5	17.0	3.0	16.0	6.3

Raw Data

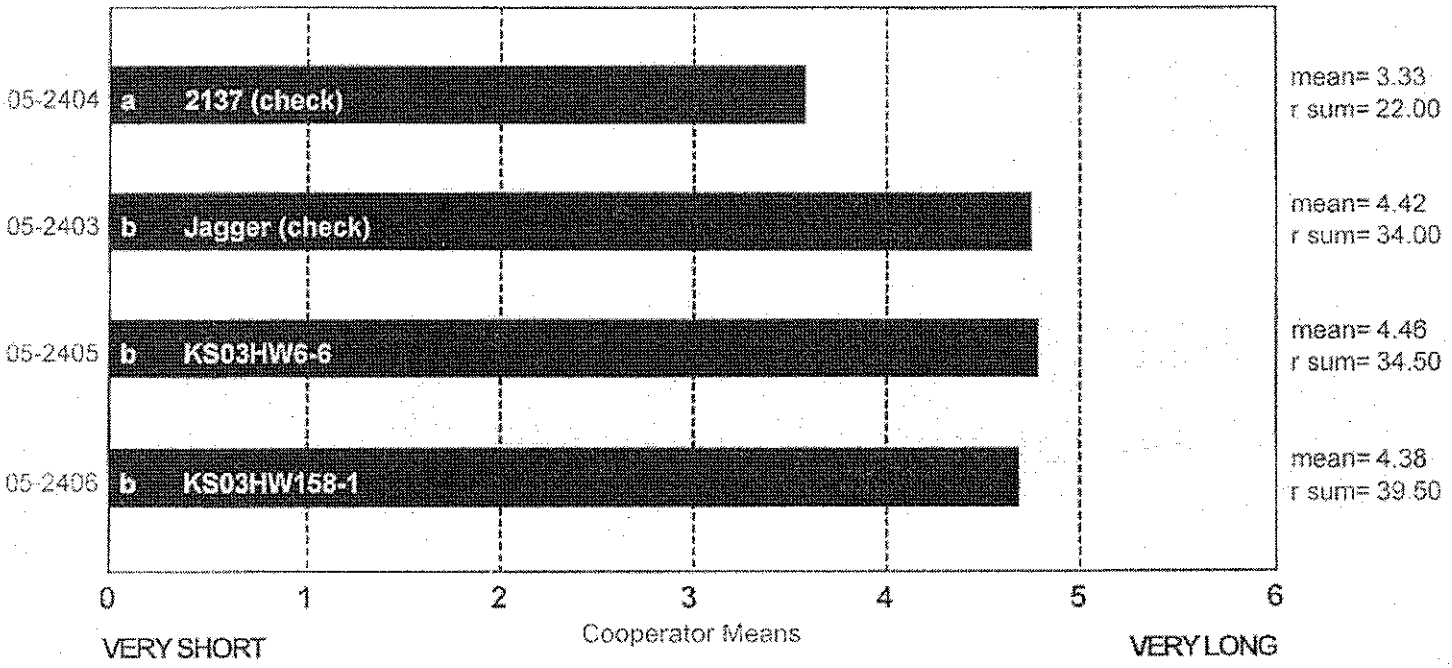
2007002244

BAKE MIX TIME

(Small Scale) Kansas-Hays

ncoop= 13
chisq= 7.64
chisqc= 11.82
cvchisq= 7.82
crdiff= 9.32

Variety order by rank sum.
Samples with the same letter not different at 5.0% level of significance.

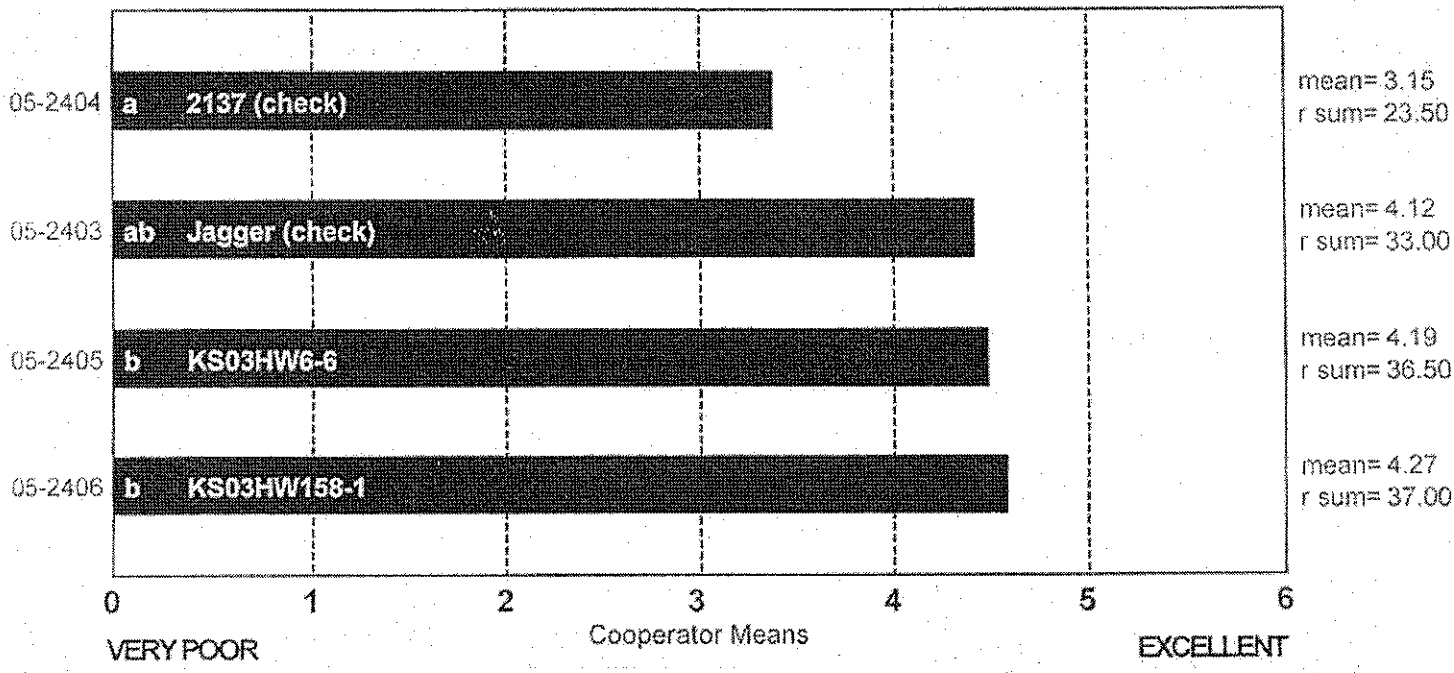


MIXING TOLERANCE

(Small Scale) Kansas-Hays

ncoop= 13
chisq= 5.42
chisqc= 8.49
cvchisq= 7.82
crdiff= 9.82

Variety order by rank sum.
Samples with the same letter not different at 5.0% level of significance.



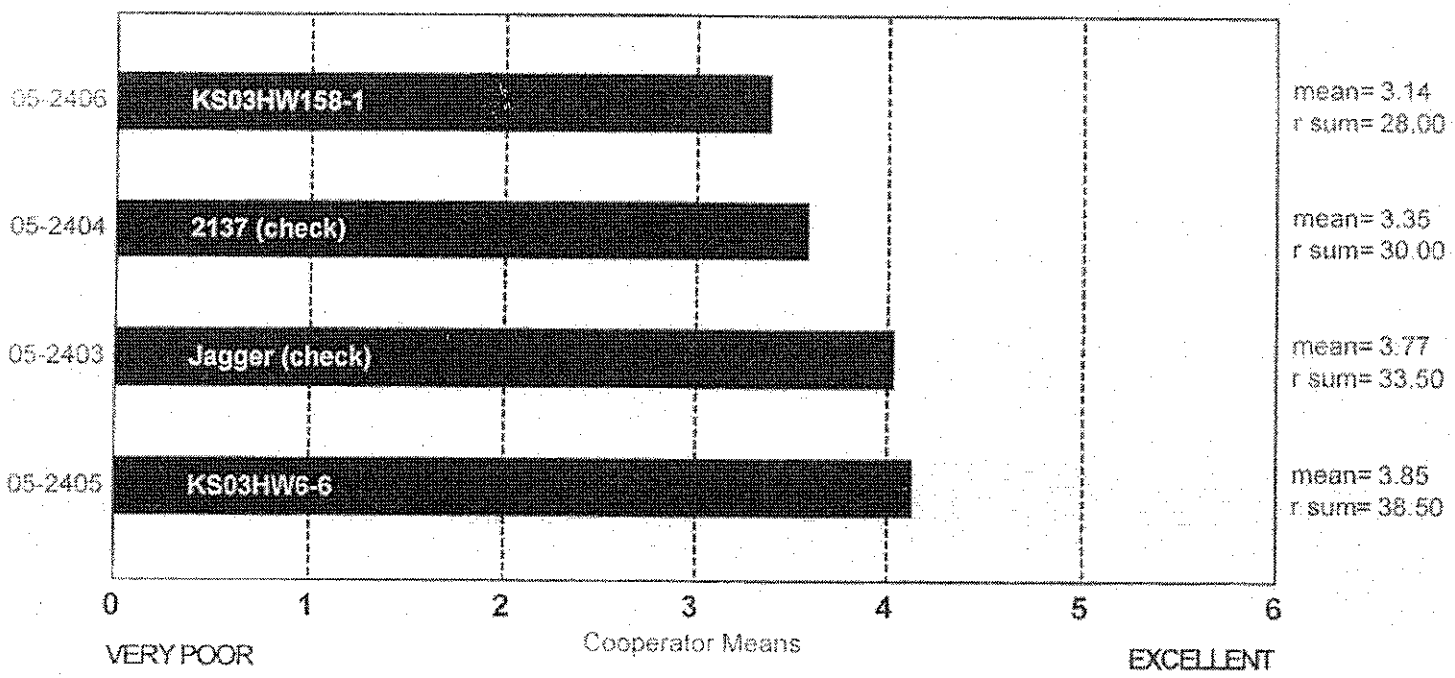
2007002444

DOUGH CHAR. 'OUT OF MIXER'

(Small Scale) Kansas-Hays

ncoop= 13
chisq= 2.93
chisqc= 4.05
cvchisq= 7.82
crdiff=

Variety order by rank sum.
No samples different at 5.0% level of significance.



DOUGH CHAR. 'OUT OF MIXER', DESCRIBED

(Small Scale) Kansas-Hays

	Sticky	Wet	Tough	Good	Excellent
05-2403 Jagger (check)	0	0	3	8	2
05-2404 2137 (check)	2	0	4	7	0
05-2405 KS03HW6-6	0	0	4	6	2
05-2406 KS03HW158-1	2	0	6	5	0

Frequency Table

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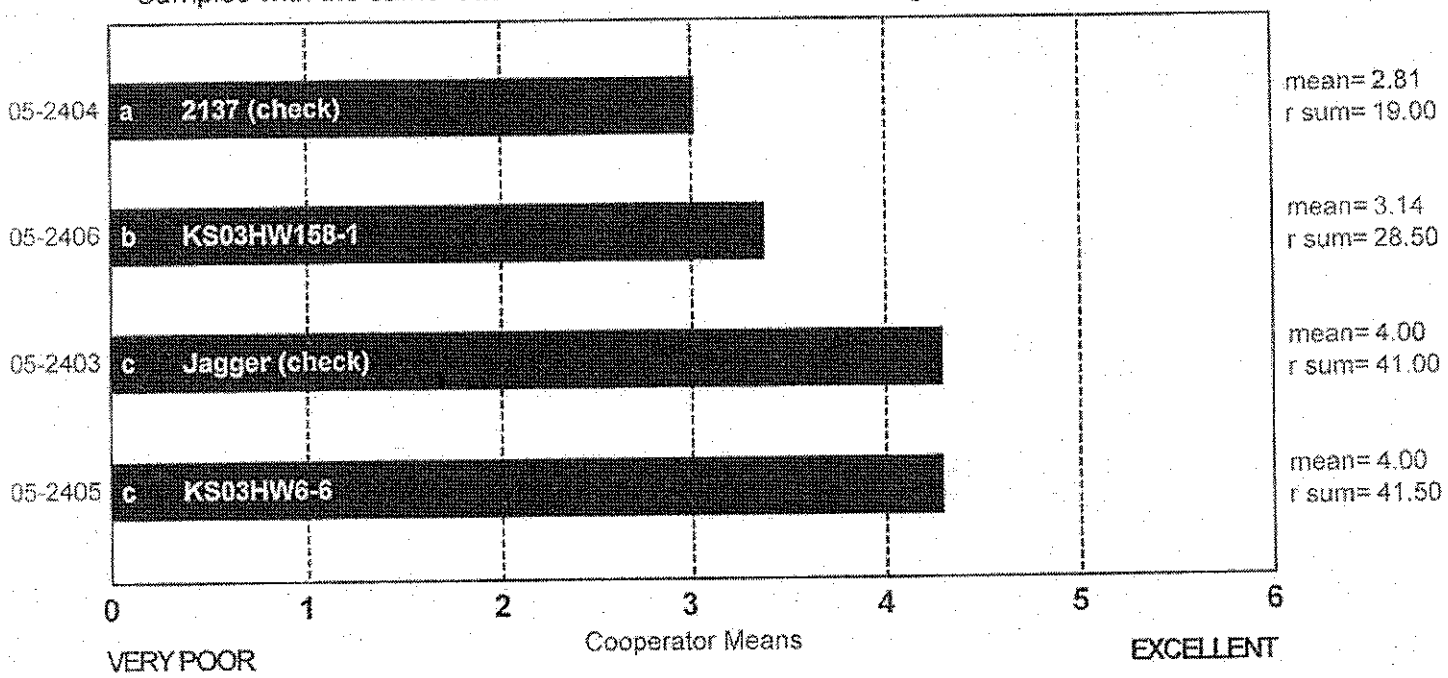
2007002244

DOUGH CHAR. 'AT MAKE UP'

(Small Scale) Kansas-Hays

ncoop= 13
chisq= 16.22
chisqc= 20.09
cvchisq= 7.82
crdiff= 8.70

Variety order by rank sum.
Samples with the same letter not different at 5.0% level of significance.



DOUGH CHAR. 'AT MAKE UP', DESCRIBED

(Small Scale) Kansas-Hays

	Sticky	Wet	Tough	Good	Excellent
05-2403 Jagger (check)	0	0	3	9	1
05-2404 2137 (check)	3	1	4	5	0
05-2405 KS03HW6-6	0	0	2	9	2
05-2406 KS03HW158-1	1	1	4	7	0

Frequency Table

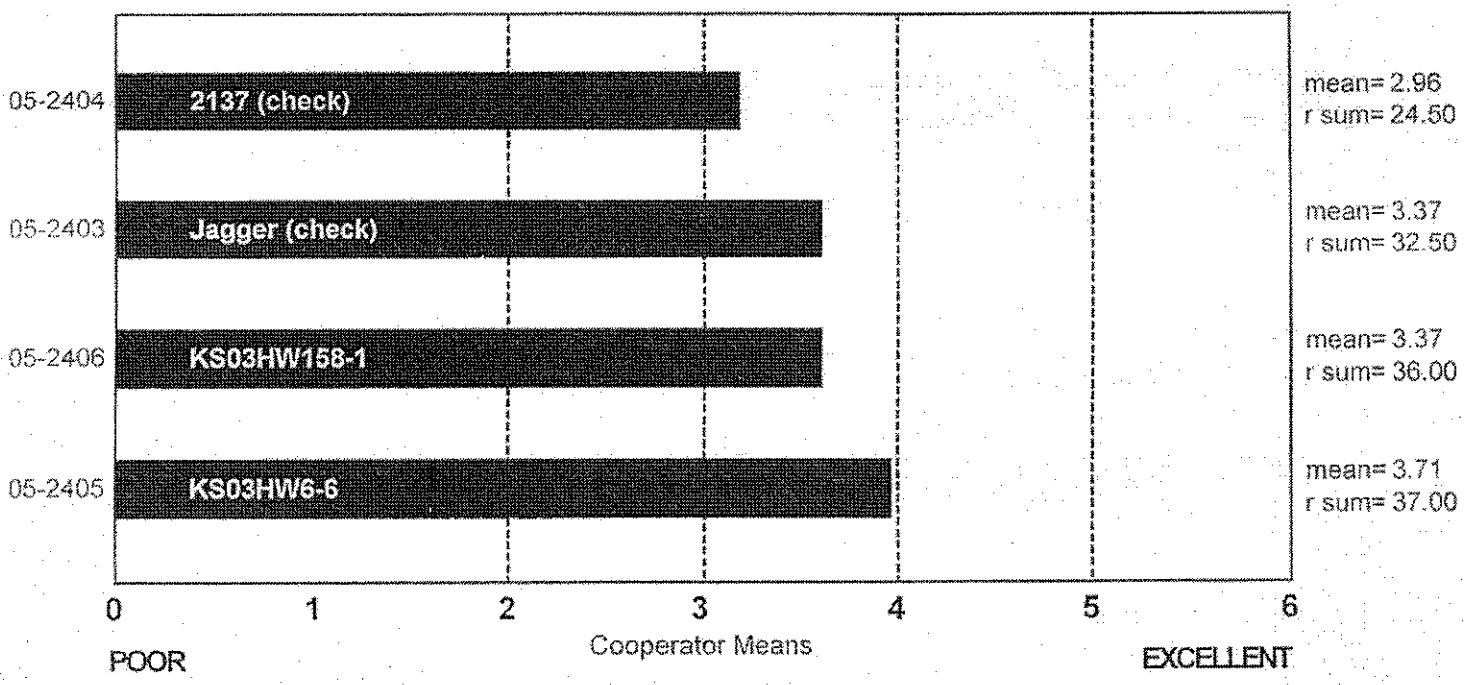
200700244

CRUMB GRAIN

(Small Scale) Kansas-Hays

ncoop= 13
chisq= 4.45
chisqc= 5.97
cvchisq= 7.82
crdiff=

Variety order by rank sum.
No samples different at 5.0% level of significance.



CRUMB GRAIN, DESCRIBED

(Small Scale) Kansas-Hays

	Open	Fine	Dense
05-2403 Jagger (check)	5	7	1
05-2404 2137 (check)	5	4	4
05-2405 KS03HW6-6	7	3	3
05-2406 KS03HW158-1	7	5	1

Frequency Table

42

2007002244

CELL SHAPE, DESCRIBED

(Small Scale) Kansas-Hays

	Round	Irregular	Elongated
05-2403 Jagger (check)	0	10	3
05-2404 2137 (check)	4	7	2
05-2405 KS03HW6-6	3	7	3
05-2406 KS03HW158-1	3	6	4

Frequency Table

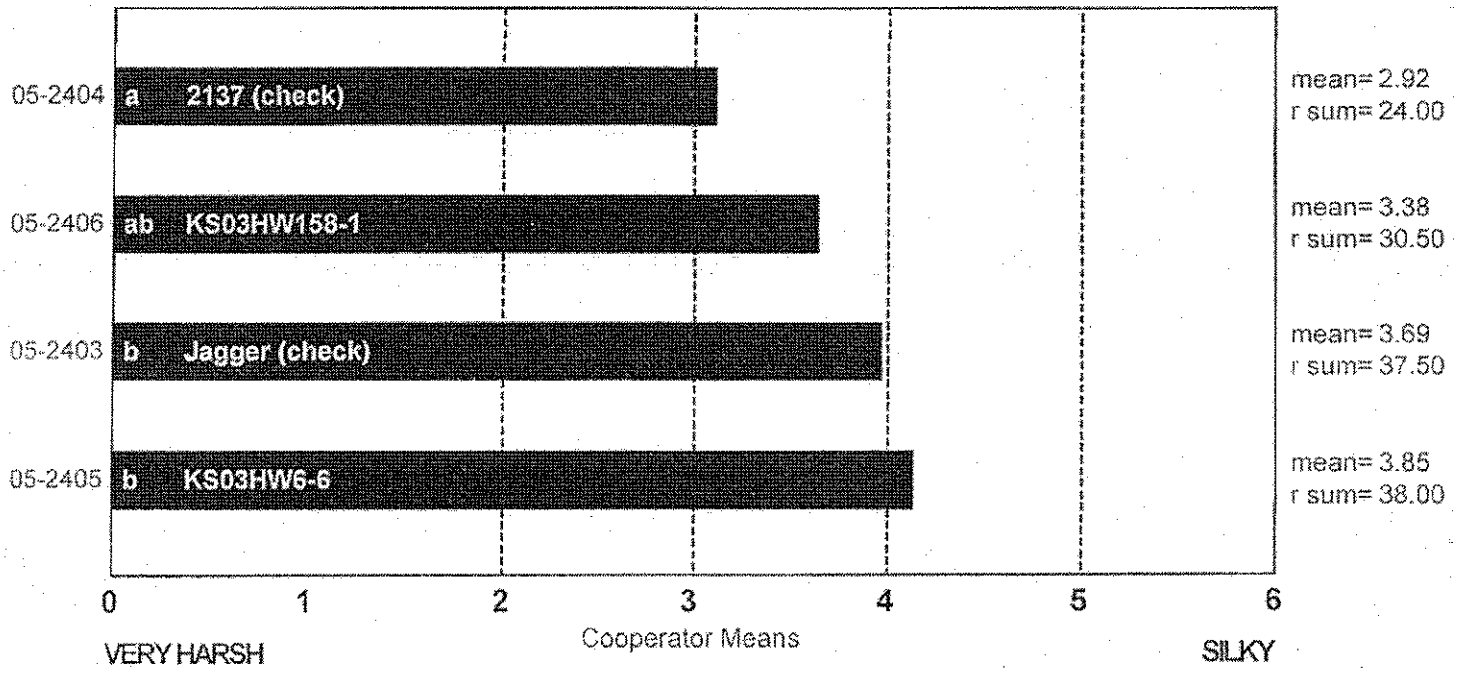
200700244

CRUMB TEXTURE

(Small Scale) Kansas-Hays

ncoop= 13
chisq= 6.07
chisqc= 10.81
cvchisq= 7.82
crdiff= 8.85

Variety order by rank sum.
Samples with the same letter not different at 5.0% level of significance.



CRUMB TEXTURE, DESCRIBED

(Small Scale) Kansas-Hays

	Harsh	Smooth	Silky
05-2403 Jagger (check)	2	8	3
05-2404 2137 (check)	6	6	1
05-2405 KS03HW6-6	3	4	6
05-2406 KS03HW158-1	4	6	3

Frequency Table

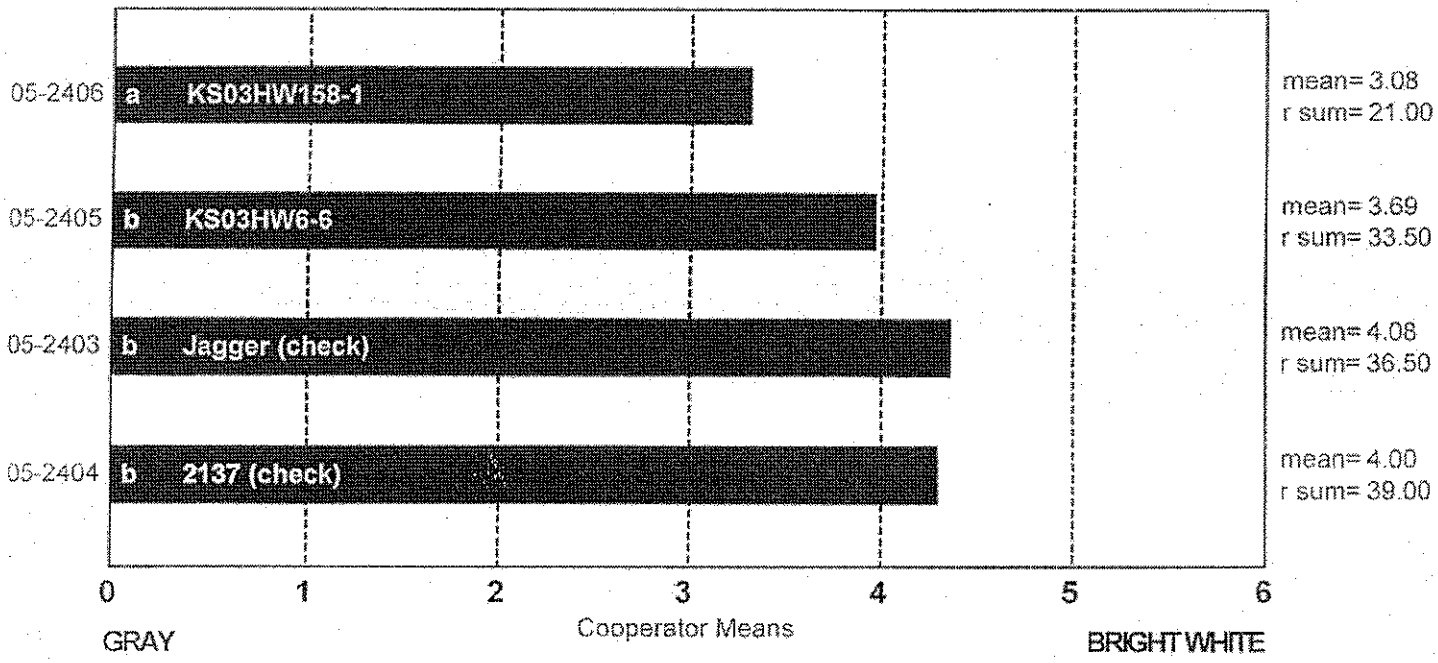
2007002444

CRUMB COLOR

(Small Scale) Kansas-Hays

ncoop= 13
chisq= 8.84
chisqc= 13.36
cvchisq= 7.82
crdiff= 9.16

Variety order by rank sum.
Samples with the same letter not different at 5.0% level of significance.



CRUMB COLOR, DESCRIBED

(Small Scale) Kansas-Hays

	Gray	Yellow	Dull	Creamy	Bright White
05-2403 Jagger (check)	0	0	0	12	0
05-2404 2137 (check)	1	0	1	6	0
05-2405 KS03HW6-6	1	0	2	8	0
05-2406 KS03HW158-1	0	4	4	5	0

Frequency Table

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LOAF WEIGHT, ACTUAL

(Small Scale) Kansas-Hays

	Coop. A	Coop. B	Coop. C	Coop. D	Coop. E	Coop. F	Coop. G	Coop. H	Coop. I	Coop. J	Coop. K	Coop. L	Coop. M
05-2403 Jagger (check)	142.7	462.7	495.0	139.3	142.4	155.5	471.0	458.0		432.0		418.6	147.1
05-2404 2137 (check)	141.4	465.6	495.0	141.8	140.5	158.3	477.0	464.0		436.0		419.1	144.0
05-2405 KS03HW6-6	142.8	462.7	490.0	138.0	139.7	155.7	472.0	456.0		435.0		417.6	144.7
05-2406 KS03HW158-1	141.4	465.8	490.0	139.1	143.4	157.2	475.0	462.3		433.0		418.4	146.2

LOAF VOLUME, ACTUAL

(Small Scale) Kansas-Hays

	Coop. A	Coop. B	Coop. C	Coop. D	Coop. E	Coop. F	Coop. G	Coop. H	Coop. I	Coop. J	Coop. K	Coop. L	Coop. M
05-2403 Jagger (check)	910	2663	2900	800	920	878	2875	2853	900	2800	908	2400	898
05-2404 2137 (check)	840	2588	2800	625	750	753	2650	2956	785	2500	790	2450	783
05-2405 KS03HW6-6	1020	2688	3150	775	940	958	3000	2691	960	2700	967	2300	925
05-2406 KS03HW158-1	940	2688	3050	725	920	895	2750	2897	875	2700	862	2350	905

Raw Data

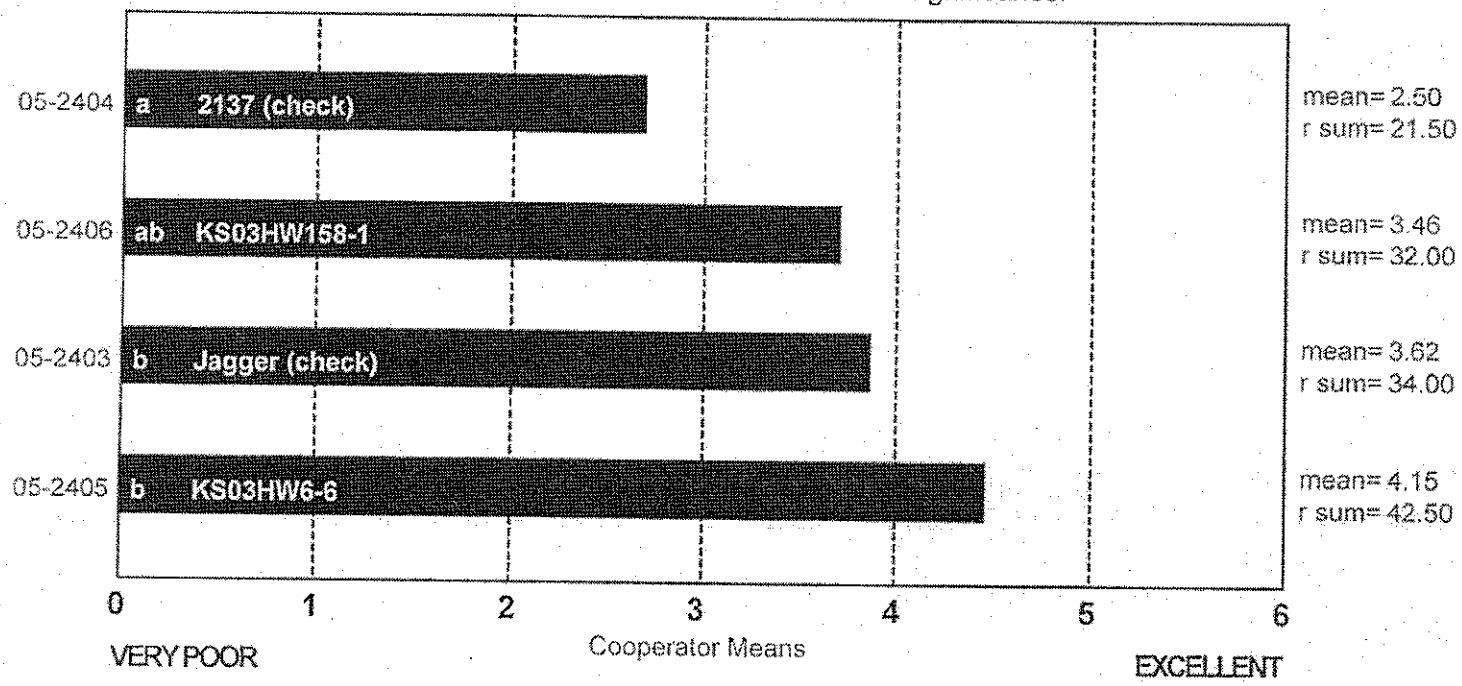
LOAF VOLUME

2007002444

(Small Scale) Kansas-Hays

ncoop= 13
chisq= 10.32
chisqc= 12.08
cvchisq= 7.82
crdiff= 10.67

Variety order by rank sum.
Samples with the same letter not different at 5.0% level of significance.

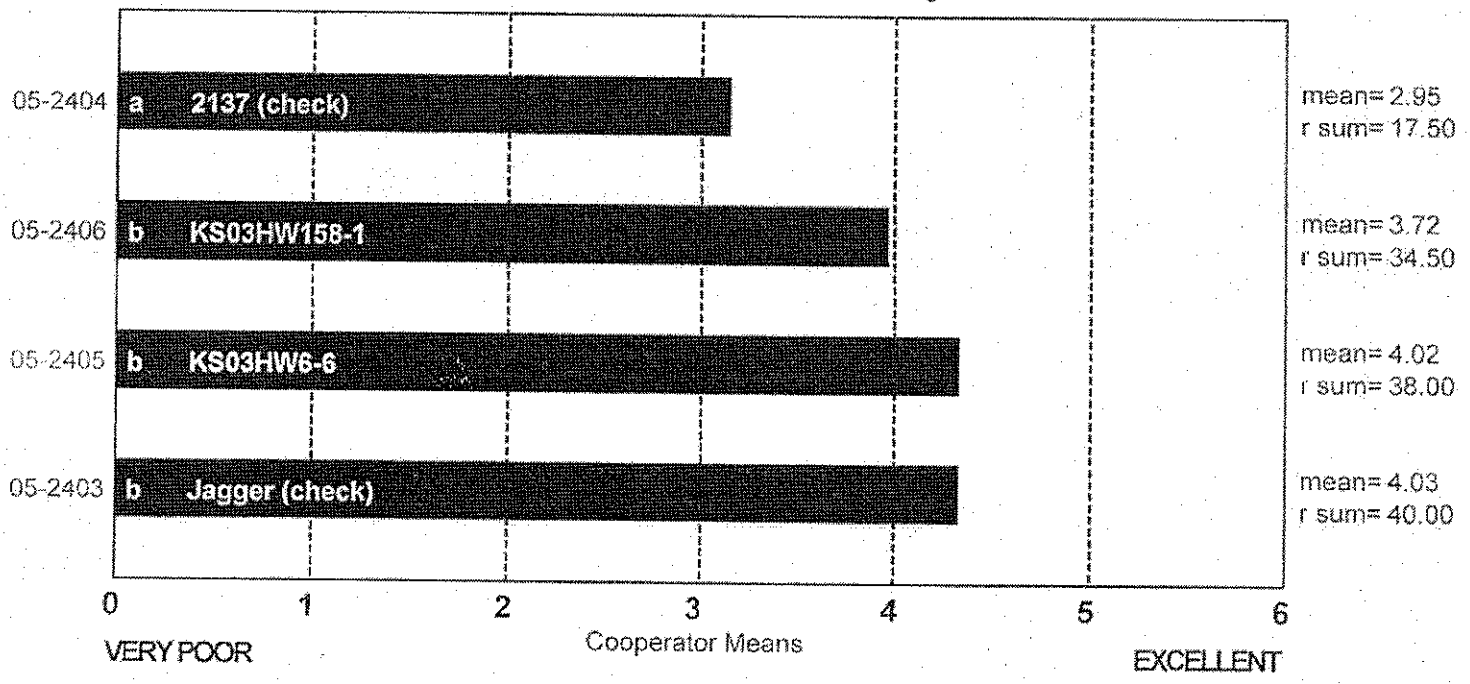


OVERALL BAKING QUALITY

(Small Scale) Kansas-Hays

ncoop= 13
chisq= 14.56
chisqc= 16.90
cvchisq= 7.82
crdiff= 9.71

Variety order by rank sum.
Samples with the same letter not different at 5.0% level of significance.



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COOPERATOR'S COMMENTS 200700244

(Small Scale) Kansas-Hays

COOP. **05-2403 (Jagger (check))**

A. Nice crumb.
B. High absorption, average in most categories.
C. No comments.
D. No comments.
E. Good absorption, good quality.
F. Low volume for protein, weak dough.
G. No comments.
H. Good strength and good absorption.
I. Good loaf volume, nice out of mixer, slightly harsh crumb texture.
J. Several doughs could have mixed longer.
K. Strong dough, nice white color, good absorption, and good tolerance.
L. No comments.
M. Good flour protein, good absorption, slight long mix tolerance, good crumb grain, low loaf volume.

COOP. **05-2404 (2137 (check))**

A. No comment.
B. Good absorption, very short mix time, very short mixing tolerance, very fine grain, white crumb.
C. Low absorption.
D. No comment.
E. Questionable quality, very low loaf volume, poor break and shred.
F. Low volume for protein and poor grain.
G. No comment.
H. Very open grain, harsh texture, good volume.
I. Creamy crumb color, low loaf volume.
J. No comment.
K. Poor gluten development, small volume, dense dough.
L. No comment.
M. Low absorption, poor tolerance, low loaf volume, but crumb grain satisfactory.

COOPERATOR'S COMMENTS
(Small Scale) Kansas-Hays (continued)

COOP. 05-2405 (KS03HW6-6)

A. Good volume for protein.
B. Good absorption, average in most categories.
C. Low absorption, good mix tolerance, and good volume.
D. No comment.
E. Acceptable quality.
F. Solid performance, good volume for protein.
G. No comment.
H. Low volume, good mix strength.
I. Excellent handling, smooth crumb texture and white crumb color, good loaf volume.
J. 2410, 2411, 2413, 2418, and 2420 – loaves had squatty appearance and low.
K. Outstanding bake performance and mix tolerance, long mix stability.
L. No comment.
M. Good tolerance, good dough feel, good crumb grain, and low volume.

COOP. 05-2406 (KS03HW158-1)

A. Nice crumb.
B. Good absorption, good grain, and yellow crumb color.
C. Poor color.
D. No comment.
E. Good absorption and mixing tolerance, however, slightly sticky out of mixer.
F. Good dough.
G. No comment.
H. Good mix strength, above average interior, average volume.
I. Harsh crumb, and yellow crumb color.
J. Good volume.
K. Small volumes, dense doughs, and dull color.
L. No comment.
M. Long mix tolerance, all other ratings good.

Notes: **B, H, J, K, and L** comments based on sponge and dough bake test.

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U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL MARKETING SERVICE

EXHIBIT E
STATEMENT OF THE BASIS OF OWNERSHIP

Application is required in order to determine if a plant variety protection certificate is to be issued *(7 U.S.C. 2421)*. The information is held confidential until the certificate is issued *(7 U.S.C. 2426)*.

1. NAME OF APPLICANT(S)	2. TEMPORARY DESIGNATION OR EXPERIMENTAL NUMBER	3. VARIETY NAME
Kansas Agricultural Expermient Station	KS03HW158	RonL
4. ADDRESS *(Street and No., or R.F.D. No., City, State, and ZIP, and Country)*	5. TELEPHONE *(Include area code)*	6. FAX *(Include area code)*
Kansas State University Waters Hall Manhattan KS 66506	785.532.6147	785.532.6563
	7. PVPO NUMBER	#200700244

8. Does the applicant own all rights to the variety? Mark an "X" in the appropriate block. **If no, please explain.** ☑ YES ☐ NO

9. Is the applicant (individual or company) a U.S. national or a U.S. based company? **If no, give name of country.** ☒ YES ☐ NO

10. Is the applicant the original owner? ☒ YES ☐ NO **If no, please answer one of the following:**

a. If the original rights to variety were owned by individual(s), is (are) the original owner(s) a U.S. National(s)?
☐ YES ☐ NO **If no, give name of country**

b. If the original rights to variety were owned by a company(ies), is (are) the original owner(s) a U.S. based company?
☐ YES ☐ NO **If no, give name of country**

11. Additional explanation on ownership *(Trace ownership from original breeder to current owner. Use the reverse for extra space if needed)*:

PLEASE NOTE:

Plant variety protection can only be afforded to the owners (not licensees) who meet the following criteria:

1. If the rights to the variety are owned by the original breeder, that person must be a U.S. national, national of a UPOV member country, or national of a country which affords similar protection to nationals of the U.S. for the same genus and species.

2. If the rights to the variety are owned by the company which employed the original breeder(s), the company must be U.S. based, owned by nationals of a UPOV member country, or owned by nationals of a country which affords similar protection to nationals of the U.S. for the same genus and species.

3. If the applicant is an owner who is not the original owner, both the original owner and the applicant must meet one of the above criteria.

The original breeder/owner may be the individual or company who directed the final breeding. See Section 41(a)(2) of the Plant Variety Protection Act for definitions.

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL MARKETING SERVICE
SCIENCE AND TECHNOLOGY
PLANT VARIETY PROTECTION OFFICE
BELTSVILLE, MD 20705

EXHIBIT F
DECLARATION REGARDING DEPOSIT

NAME OF OWNER (S)	ADDRESS *(Street and No. or RD No., City, State, and Zip Code and Country)*	TEMPORARY OR EXPERIMENTAL DESIGNATION
Dr. Forrest Chumley	Kansas Agricultural Experiment Station Kansas State University 148 Waters Hall Manhattan, KS 66506-4008	KS03HW158 VARIETY NAME RonL
NAME OF OWNER REPRESENTATIVE (S)	ADDRESS *(Street and No. or RD No., City, State, and Zip Code and Country)*	FOR OFFICIAL USE ONLY PVPO NUMBER
Dr. T. Joe Martin	KSU Agricultural Center–Hays 1232 240th Avenue Hays, KS 67601-9228	#200700244

I do hereby declare that during the life of the certificate a viable sample of propagating material of the subject variety will be deposited, and replenished as needed periodically, in a public repository in the United States in accordance with the regulations established by the Plant Variety Protection Office.

T. Joe Martin
Signature

3-19-07
Date